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SDSU

FOREWORD

I am pleased to introduce you to Volume 3 of the *Journal of Undergraduate Research.* In Volumes 1 and 2 nine different departments, twenty-two different students, and eleven different faculty members were represented. In Volume 3 these numbers are, of course, expanded. The previous volumes' articles have ranged from a scholarly analysis of *Uncle Tom's Cabin* to a very technical report on genetics in plant science. What this variety tells us is that undergraduate scholarship and research are "alive and well" across the entire University and its colleges.

South Dakota State University faculty members are to be commended for their diligence in fostering undergraduate research. It takes determination, time, and close interaction with students. Our students are to be praised for once again demonstrating their competitive excellence, which means they will compete well beyond SDSU. Undergraduate Research opportunities help to prepare them to "go anywhere from here."

The facts and details that our student learn will grow obsolete fast and be easily forgotten. Hopefully the concepts and principles that undergraduates absorb and master will stay longer in their content base. However, the most lasting from their undergraduate experiences are the processes (either intellectual or scientific) that they learn. Scholarship and research are among the most relevant of these processes.

The learning experiences manifested in the articles in Volume 3 of the *Journal of Undergraduate Research* will not be forgotten. Processes learned can be applied over and over and set the stage far more complex learning in the future. Faculty members and students represented in this volume are to be praised for their efforts to learn/teach and demonstrate the research process in a number of different fields.

Carol J. Peterson, Ph.D.
Provost and Vice President for Academic Affairs
Professor of Nursing

Table of Contents

SDSU Journal of Undergraduate Research

(Next deadline for submission: June 1, 2006)

MANUSCRIPT SUBMISSION GUIDELINES

This document is intended to provide you with some guidance regarding the final structure and format your research manuscript should possess to warrant publication in the SDSU *Journal of Undergraduate Research*. Student authors wishing to have their work published in the *Journal* are advised to follow these guidelines as closely as possible, as manuscripts submitted to the *Journal* that are not of high quality in content and format may be rejected by the editor. The Journal editor does understand that research products and manuscripts from different disciplines may take on quite different forms. As such, if these guidelines do not adequately answer your questions, simply follow the format and guidelines utilized by a major scholarly journal in your field of study. **Professional journal articles in your field of study are a guideline for manuscript length.** (When in doubt, article conciseness is important). Your faculty mentor should be able to advise you in this regard.

If these guidelines do not answer your questions, or if additional questions about content and format arise, please do not hesitate to contact the journal editor, George Langelett, Economics Department, 688-4865, or George.Langelett@sdstate.edu.

SUBMISSION: HARD COPY AND ELECTRONIC FORMATS

Your JUR manuscript must be submitted in both a hard copy (printed) form **AND** an electronic format through email.

All manuscripts must be submitted to the JUR by your faculty mentor. Manuscripts submitted by students will not be accepted for publication. All manuscripts should be submitted to the *Journal of Undergraduate Research*, Administration Building room 130. Electronic versions should be sent via email to Linda.Winkler@sdstate.edu by the specified deadline (next year is June 1, 2006). Every effort will be made by the editorial staff to reproduce your manuscript in a form that closely resembles your hard copy. However, some slight variations may occur in converting your files to those used by the publisher. Your careful adherence to the information in the next few paragraphs will ensure that your manuscript is reproduced with minimal errors.

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If graphics are to be included, they must also be submitted as separate black and white files. Color images are accepted only for those who are willing to pay the extra cost to print color. All digital art or other representations must be saved as either EPS, TIF orJPG files with a resolution of no less than 300 dpi (dots per inch) for best quality reproduction. The Editor will insert these into the proper positions within your *Journal* article later. Finally, be aware that Word Publisher artwork cannot be translated and will be useless to the editorial staff.

MANUSCRIPT REVIEW

After your manuscript has been submitted to the *SDSU Journal of Undergraduate Research*, it will be reviewed by the editorial staff, and, if deemed acceptable for publication, converted into a "publication-ready" format ("proof"). A hard copy of the manuscript proof will then be returned to your faculty mentor by July 1 for final review. At that time, it will be your mentor's responsibility to make any final changes to the document and return it to the Editors by the noted deadline (July 15). It is imperative that all proofs be returned to the *Journal* staff in a timely manner so that any final changes can be incorporated before the volume goes to press.

FINAL PRODUCT

The final form of your paper will depend greatly on the nature of your topic and certain publishing conventions that may exist within your discipline. It is expected that the faculty advisor for each project will provide substantial guidance in this matter. An excellent general resource providing details of the content, style, and organization of a typical journal article is the *Publication Manual of the American Psychological Association*, which is accepted as a definitive source in many disciplines. While the emphasis there is on empirical research reports (based upon original research and data collection), other types of papers are also described (review articles, theoretical articles), and an appendix: "Material Other Than Journal Articles," may be useful.

Your discipline may have its own publication style preferences, and you should explore this matter with your faculty advisor. For most all disciplines, however, articles should follow a standard format and begin with a descriptive title, the name of the author(s) the name(s) of the faculty advisor(s), and an abstract describing in brief the purpose, methodology, and findings or conclusions of the project (see below). Manuscripts describing empirical research will typically be organized into further subsections, labeled Introduction, Method, Results, Discussion, (or variations on those subheadings), along with a complete list of References.

The rest of these guidelines are intended to provide you with a sense of the appearance and content of a typical final research report, as it should appear in the *SDSU JUR*. Beginning with "Title of Your Article" below, the remainder of this document is written in the *SDSU JUR* style. Please note font sizes, format, and section content, and use this example to guide you.

*** Sample Format ***
Title Of Your Article
Author(s): Your Name, Your Partner's Name(s)
Faculty Sponsor: Faculty Mentor's Name
Department: Economics

ABSTRACT

This will be a brief statement of what was done in your research, along with your principal results and conclusions. Only the most important facts should be related here, in non-indented paragraph form. Offset the abstract by using margins that are indented 0.5" on each side relative to the body of your manuscript. You may list key words to aid in on-line computer-search applications, if that is appropriate. For example, **Keywords**: undergraduate research, manuscript, submission, guidelines.

INTRODUCTION

This is the first formal section of a research report. This and the sections to follow should be single-spaced and laser-printed on only one side of the paper (8.5" x 11"). Early in this section, provide a general description of the research problem or activity. Attempt to identify and define whatever terms your reader will need to understand your project. The remaining paragraphs are often used to summarize relevant findings from previously completed research. Always be sure to cite your sources. Sarbin and Coe (1969) state that "in preparing a . . . report, the student must pay careful attention to the problems of documentation." In these examples of citations, "the documentation is contained in the parentheses . . ." (Sarbin and Coe, 1969). To find the remaining information, the reader examines the reference list at the end of the paper. This citation style is sometimes called "scientific notation." Other citation styles may be more appropriate to your own disciplines. Be sure to be consistent, and to discuss this with your faculty advisor. Ultimately, you should use a citation style that is commonly accepted within your discipline.

The last portion of an introduction is often used to state the specific expected outcomes of the project; sometimes this appears as one or more formal testable hypotheses.

METHODS

The content of this subsection may vary greatly, depending upon the nature of the research project. You should refer to publication manuals or published research for information specific to your type of project. Sometimes this sub-section is labeled "Materials and Method." Figures (see below) are often used to clarify and explain important details. In general you should use this sub-section to explain to your reader, in as clear a way as possible, what you did, in the order that you did it. In an empirical research report, you should try to provide enough detail that another researcher could essentially duplicate your study without referring excessively to other sources.

RESULTS

This should be a clear description of any data (or other material) generated as a result of your research. It must start out as a written description, but this subsection is often supplemented with FIGURES and TABLES, or PLATES, or other types of graphic images. These are never sufficient by themselves. Figures and Tables should not appear in your paper until after they've been mentioned or referred to in the written portion of this section. They should appear as soon as is reasonable after such mention, either on the same page, or on the next page (see Figure 1, and Table 1). Notice, in particular, that in most scientific papers, the number and title of a Table appear above the data being described, but the number and title of a Figure appear below the data. Any units of measure must appear either in the title, or independently in the column or row headings. A table is useless unless the reader can understand exactly what is represented. **Graphic materials, properly labeled, should be included IN THE BODY of your paper, not grouped at the end.** (See the above section labeled "Submission" for further details.)

The Results section is also the place to include any statistical interpretation of the data, if such exists. Be sure to point out any important features of your findings, but AVOID to the extent possible, any THEORETICAL INTERPRETATION unless you are combining this with the next section (DISCUSSION or CONCLUSIONS).

DISCUSSION (AND/OR CONCLUSIONS)

This section is sometimes combined with the previous RESULTS section, especially when that permits a more efficient presentation. Your "Discussion" should include any theoretical interpretation of your data, including, when appropriate to your topic, the following: (1) WHETHER your results support any

specific hypothesis or hypotheses you may have stated in your introduction; (2) HOW your results compare with the results in your cited research sources; and (3) WHAT theories or explanations seem to best explain or account for the results that you are describing.

Again, be sure to cite (Sarbin and Coe, 1969) the sources for theoretical ideas and explanations provided by other writers or sources. Also, address whether there any practical applications for the results or methods used in your research.

LIMITATIONS

It is often useful, particularly in undergraduate research, to provide a summary of the limitations of the research from methodological, theoretical or other points of view, to provide perspective and to serve as a possible basis for improvements in future projects.

ACKNOWLEDGEMENTS

Feel free to use this section to BRIEFLY acknowledge any and all who helped you bring your project through to fruition. You may also thank any funding sources if appropriate.

REFERENCES

Provide a complete list of all cited materials, in a format that is consistent with publications in your area of study.

APPENDIX

This section is optional, and generally unnecessary. In some cases, it may be included to provide a more complete description of materials used. The Editor of the *SDSU Journal of Undergraduate Research* would prefer that no appendices be used. However, if absolutely necessary, the number of pages in an appendix should be kept to an absolute minimum!

The Journal of Undergraduate Research would like to thank the Golden Key International Honour Society for their efforts to promote undergraduate research at South Dakota State University.

South Dakota State University Students and Debt Management

Authors: Katherine Delaney
Tamara Sinning
Ashley Berndt
Faculty Sponsor: Dr. George Langelett
Department: Economics

STATEMENT OF PURPOSE

Money! It comes in slowly and disappears faster than students ever thought possible. People are falling into more debt than ever before, especially college students. Where does all the hard-earned money go? How can students develop habits that help them keep track of their spending and not fall deep into debt? The purpose of our research is to investigate how money can be used wisely and how to make good financial spending decisions. College students' debt behavior is important because today's students are the workforce of tomorrow. A plan is needed to help students learn how to handle today's financial challenges. College tuition and expenses rise annually and many students spend more money than they earn, which leaves them struggling to manage their debt. The purpose of this paper is to study how college students manage their debts. Most students take out student loans to get a college degree; therefore they have the potential be $40,000 in debt. This debt does not include any credit card debt they might have acquired while in college. If students do not learn the proper way to manage their debt, they may be hurt financially. They may have trouble buying a house or even a car.

REVIEW OF LITERATURE

Most Americans have debt. Yet many Americans try not to think about just how much debt they have (Fowles, 2004). To get out from the burden of debt, one must face the uncomfortable truth that it may take them 30 years to pay off a credit card balance of just $5000.

Most debtors do not know the warning signs of too much debt. Credit is great when it is used wisely, but one can get in too deep if debt is not kept on track, leading one into financial ruin. Deborah Fowles, author of *Your Guide to Financial Planning*, gives these warning signs for people to consider.

You can tell that you are over your head if: You do not have any savings, you make minimum payments on your credit cards; you use credit cards for things you used to buy with cash (such as groceries); you use increasing amounts of you total income to pay off debts; you have more than two or three major credit cards; after you pay your credit card bills—you increase your balance by the same amount; you are at or near your credit

limit; you are unsure of the total amount that you owe on all of your debts, you take out cash advances on your credit card to pay other bills, you have tried to make purchase, and your credit card has been denied; you have been denied credit; you bounce checks; you lie to your family members about the spending or you hide purchases; and one of the final warning signs of too much debt is that you are getting calls from bill collectors (Fowles).

When evaluating college students, it has been found that 80% of all college students use credit cards (Richards, 1998). "Even though students have little spending money, credit card companies have seized the college market" (Richards, 1998). Having all of these funds available at their fingertips can create a debt management problem. When surveyed, 60-67% of college graduates have student loans. Their average payment on student loans was $222 per month for men and $141 per month for women (Collegiate Funding Service). The average credit card debt per student has risen from $1,879.00 in 1998 to $2,327 in 2001 (*The Brookings Register*). So, not only do college students 'graduate' into debt due to student loans, but also, the spending habits adopted while in college create more debt for them. Thus, it is essential that we investigate how students manage their debt while in college.

STATISTICAL METHODOLOGY

Research for this paper was carried out according to standard sampling methodology. Surveys were conducted using a random sample. South Dakota State University students were the target population. The random sample was chosen by randomly selecting a course from the course listing book. The course was a two-hundred level economics class on campus, with around 90 students enrolled. (The course was a macro economics course and did not cover any personal finance topics). The survey was developed to contain nineteen questions. The respondents were asked questions that focused on how they were paying for college, their yearly income, debt management techniques, budgeting, savings, and credit card awareness. The final questions of the survey focused specifically on general demographics such as: age, sex, and marital status. A combination of yes/no and multiple choice questions were used. The survey was distributed at the beginning of a class period for the course selected and collected immediately following respondent completion. (See Appendix A for a sample of the survey instrument.)

Seventy-eight surveys were received. Four surveys were found to be incomplete; therefore, they were discarded and not evaluated with the rest. Seventy-four surveys were coded and put into a data base. The surveys were coded using numerical values. Questions that were answered 'yes' were equal to one, while questions that were answered 'no' were equal to zero. Gender was coded using one for male and a zero for female. All questions that contained values were coded according to the highest chosen

value. Evaluation was then conducted using the SPSS program. Many different regressions, cross tab, and frequency tables were used.

Demographics were also evaluated. Thirty-eight males and 36 females participated in the survey. Fifty-six percent of the students that took the survey were sophomores at South Dakota State University.

FINDINGS

Questions were analyzed using a linear regression model. These results are presented in Table 1. Significant t-scores tell a story of how each question relates to one of the four explanatory values used. The explanatory variables that hold some significance are the student's age (year in college), gender, income, and the total number of credit hours for which they are currently enrolled.

Explanatory Variables	Pay for College by Working	Manage Debt with a Budget	Manage Debt with Credit Cards	Manage Debt by Other Means
Year in College	B = .078 (t) 1.103	B = -.091 (t) -1.09	B = .059 (t) 1.72 *	B = -.005 (t) -.075
Gender	B = .294 (t) 2.81 **	B = -.231 (t) -1.87 *	B = .092 (t) 1.81 *	B = .271 (t) 2.86 ***
Yearly Income	B = -.012 (t) -.715	B = -.231 (t) -1.87 *	B = -.019 (t) -2.38 **	B = -.030 (t) -2.00**
# of Credits	B = -.022 (t) -1.19	B = .011 (t) .500	B = .005 (t) .561	B =-.020 (t) -1.21
Constant	B =.283	B = .256	B = -.115	B = .533
R Squared	.141	.111	.116	.177

Table 1

First, in the pay-for-college-by-working equation, the explanatory variable that was significant was gender. The gender variable was coded with a number one for male and a zero for female. The significant value suggests that males tend to work more to pay for their college education.

The explanatory variables of significance in the manage-debt-with-a-budget equation were gender and yearly income. As stated above, gender was coded using a one for male and zero for female, while yearly income was coded according to the highest chosen value given. The significant variables suggest that females and those with lower income are more likely to use a budget.

In the manage-debt-with-credit-cards equation, all three variables were significant. Those significant variables suggest that an older student was more likely to use their credit card to manage their debt, more males than females use credit cards, and the lower a student's income, the more likely they were to use a credit card to manage their debt.

The students manage-debt-by-other-means equation was also found to hold some interesting findings. The significant variables for gender and income suggest that males and students with lower incomes are more likely to use other means to manage their debt.

Table 2 examines three more issues, namely: Do you currently use a budget, What is your total credit card debt, and Would you utilize professional debt management counseling on campus if it were free? The significant variable for using a budget was income. It suggests that higher income people are more likely to use a budget.

Explanatory Variables	Use a Budget?	Total Credit Card Debt	Seek Counseling
Year in College	B = -.077 (t) -.933	B = -.151 (t) -.915	B = .016 (t) .194
Gender	B = -.201 (t) -1.63	B =-.120 (t) -.494	B =-.140 (t) -1.1 32
Yearly Income	B = .061 (t)3.15***	B =.116 (t) 2.99***	B =.011 (t) .573
Number of Credits	B =.032 (t)1.49	B =.015 (t) .348	B = -.006 (t) -.282
Constant	B =-.105	B = .723	B =.362
R Squared	.176	.134	.022

Table 2

Yearly income, the significant variable for the students total credit card debt equation, suggests that higher income people have more credit card debt. Finally, the significant variable for those that seek counseling showed no significance at all. The fact that there was no significant variable for this question may indicate that there is no particular type of person that would seek out debt management counseling.

DISCUSSION AND IMPLICATIONS

The purpose of this research was to see how students manage their debt and to show other college students how to manage their debt. The major research question was how do students manage their debt. Findings related to gender show that males work more to pay for college and they use credit cards to manage debt. Males also use other means to manage their debt. This could include, but is not limited to, a second job or money from their parents. Females are more likely to use a budget.

The findings related to income level showed that the higher the income the more likely a person is to use a budget and that they usually carry a higher credit card debt. The lower the income the more likely the person uses a credit card to manage debt. Also, even after budgeting, the lower the income the more likely the person is to use other means to manage debt, which could include a second job. The findings indicated that no particular type of person seeks debt management counseling at South Dakota State University. Therefore, we recommend a finance management course for all incoming freshmen.

Finally, we see that men are using more credit cards to manage their debt and that women are using budgets more to manage their debt. Students need to be enrolled in financial courses not only for their future careers but so they can learn the skills they need to survive the rest of their lives.

LIMITATIONS

A limitation to this study was that the random sample of students used were enrolled in an economics class. They may have a more concrete knowledge base for financial matters and know how to manage debt. Also, some would argue that a student sample from a freshman level general class like, Mastering Lifetime Learning Skills, would respond differently than that of a 200-level economics class. Another limitation to this study was that only a class of 90 students were surveyed, which excludes the larger portion of students from South Dakota State University.

WORKS CITED

Fowles, D. (2004) You can get out of debt. Your Guide to Financial Planning. Retrieved from http://www.financialplan.about.com/cs/creditdebt.htm

Richards, Z. (1998). Colleges perpetuate exuberant credit card spending. *The Daily Athenaeum* Retrieved September 17, 2004, from http://www.wwnorton.com/uwire/news100998050.htm

Staff Writer. (2005, May 13). Students ask for personal finance class. *The Brookings Register*. Pp. A7

APPENDIX A: DEBT MANAGEMENT SURVEY

Purpose

To see how college students manage their debt. Our goal is to help other college students manage their debt.

All results are confidential. This information is only for research purposes and will only be used for class CA 31. If you have any questions, you can contact our instructor, Dr. Yao, at 605-688-5009.

(Please circle your responses.)

1. How are you paying for college?
   Savings Loans Parents
   Credit Cards Scholarships Work

2. Approximately what is your income per year? (Do not include student loans)
   $0 - $2,000 $6,001 - $8,000 $2,001 - $4,000
   $8,001 - $10,000 $4,001 - $6,000 $10,000 or more

3. How do you manage your debt?
   Budget Credit Card Savings Account Other

4. Do you currently use a budget?
   Yes No

5. Do you put money in savings each month?
   Yes No

6. How many credit cards or store charge cards do you have?
   0 1 2 3 4 5 or more

7. What is your total credit card debt?
   $0 - $1,000 $4,001 - $5,000
   $1,001 - $2,000 $5,001 - $6,000
   $2,001 - $3,000 $6,001 - $7,000
   $3,001 - $4,000 $7,000 or more

8. Do you pay more than the minimum balance on your credit cards each month?
   Yes No

9. Do you pay off your entire credit card bill each month?
   Yes No

10. When are you employed?
    Summer only School year only All year

11. Approximately how many hours a week do you work during the school year?
    0 – 10 26 – 30
    11 – 15 31 – 35
    16 – 20 36 – 40
    20 – 25

12. Which do you think is your largest expense each month?
    House/Rent Car Payment Credit Card Payment
    Cell Phone Bill Utilities Other

13. How many credits are you currently taking?
    Less then 6 6 or more 12 or more 20 or more

14. After graduation how much will you owe in student loans?
    $0 - $2,000 $6,001 - $8,000
    $2,001 - $4,000 $8,001 - $10,000
    $4,001 - $6,000 $10,000 or more

15. What year are you in college?
    Freshman Sophomore Junior Senior

16. What type of home do you live in?
    With Parents Apartment Dorm House

17. What is your Marital Status?
    Single Engaged Married

18. What is your gender?
    Male Female

19. Would you utilize professional debt management counseling on campus if it were free?
    Yes No

Color's Influence on Short-Term Memory in Each Gender

Author: Ryan W. Schroeder
Faculty Sponsor: Dr. Debra Spear
Department: Psychology

ABSTRACT

This study evaluated links between gender and short-term memory using coloration of three groups of 12 stimuli: numbers, non-emotion based words, and nonsense syllables. The participants were undergraduate students enrolled at South Dakota State University. The participants viewed these colored stimuli and recalled as many as possible. Using a between subjects design, each group of participants was assigned a specific color. When interpreted with an ANOVA and post-hoc t-tests, neither gender nor color significantly altered recall of stimuli.

COLOR'S INFLUENCE ON SHORT-TERM MEMORY IN EACH GENDER

Color is one of the most influential aspects of human perception. Color can be seen in virtually every aspect of nature and life. Colors are so significant in lives that many people have favorite colors and even favorite pieces of art based on interplay of color. Thus, color seems to be very important to people. Color is not only a perceptual detail that enhances life, but color also serves as an important function that signals and facilitates perceptual and cognitive organization.

Denby (2002) indicated that individuals who looked at a black-and-white newspaper ad had a 6% unaided recall rate whereas those who looked at the same ad in color had a 21% unaided recall rate. Kishiyama, Yonelinas, & Lazzara (2004) found that novel items, such as colored words appearing in a list of black-and-white words, increased memory recollection. In addition, Longo (2001) found that women were more likely to recall colored stimuli accurately than were men.

Unfortunately, the majority of memory studies using colored stimuli have focused on how colored pictorial stimuli influence memory and not how individual colored words or numbers influence memory. The current study will evaluate whether color has any effect on the short-term memory (when viewing numbers, non-emotion based words, and nonsense syllables) of men and women. Based on existing data, color should have little influence on short-term memory in either gender, but color should have a slightly more pronounced influence on the women's short-term memory than on the men's memory.

METHOD

Participants

Participants were 84 volunteers consisting of both men and women (32 men and 52 women). These participants were students from undergraduate South Dakota State University (SDSU) psychology classes. Most of the participants received extra credit for participating, although 11 students knowingly did not receive this credit. All participants were also entered into a drawing for free food donated by the on-campus dining service.

Students that met any of the following criterion were not allowed to participate in the study: colorblind or color deficient, memory impairments, ADHD, vision problems, reading disorders or other form of learning disability, or epilepsy. SDSU's Human Subjects Committee approved the protocol for the study and the investigator completed the National Institute of Health's online training for ethical conduct before beginning the study. In addition, the investigator informed the participants of their rights and followed the American Psychological Association's "Ethical Principles of Psychologists and Code of Conduct."

Materials

A custom-made PowerPoint presentation revealed stimuli on an overhead projector in campus classrooms. Using opportunistic sampling, the investigator assigned all the participants to groups and each group saw three PowerPoint presentations. The first presentation consisted of 12 numbers ranging from 04 to 59 arranged in no particular order. The second presentation consisted of 12 non-emotion based words, each word ranging from four to eight letters long, while the third presentation consisted of 12 sets of nonsense syllables. Three consonants arranged in an order not obviously related to words or common abbreviations constituted a nonsense syllable, such as PQG. Each of the three PowerPoint presentations (numbers, non-emotion based words, and nonsense syllables) was created in all of the selected colors: orange, red, blue, yellow, green, and black (control group), creating 18 individual PowerPoint presentations. Furthermore, each stimulus was presented on a white background, thus allowing the investigator to compare differences due to the coloration of the stimuli.

Design and Procedure

Each group, consisting of both men and women, saw all three of the PowerPoint presentations in a single color. The investigator showed all the participants a PowerPoint presentation of the twelve stimuli in 3-second intervals. After the twelfth stimulus occurred, the screen turned black and each participant received 1 minute and 15 seconds to write down all the stimuli they could remember viewing. Immediately following the 1 minute and 15 seconds, the investigator told the subjects to prepare for the next presentation.

Results

Each participant received one point for every correct number and non-emotion based word accurately written down. Because the nonsense syllables were more difficult to

accurately write down, each participant was given a half point if he or she wrote down two of the three consecutive consonants from the nonsense syllable. A participant was given one point for accurately writing down the whole nonsense syllable. The average number of non-emotion based words recalled was 7.31 (SD = 1.55) and the average number of the numbered stimuli recalled was 6.46 (SD = 1.57). However, the average number of nonsense syllables recalled, after the participants received the additional half-point increments, was 3.87 (SD = 1.31) thus indicating nonsense syllables were more difficult to recall than either the non-emotion based words or the numbers.

However, to determine the effects of color on recall of the numeric and verbal stimuli, all three stimuli groupings (numbers, non-emotion based words, and nonsense syllables) were collapsed for each participant and all the points were summed to form a single score. As shown in Figure 1, 25 men had an average recall of 17.17 (SD = 3.04) for the colored stimuli and 5 men had an average recall of 17.40 (SD = 4.25) for the control stimuli. As shown in Figure 2, 45 women had an average recall of 18.10 (SD = 2.99) for the colored stimuli and 7 women had an average recall of 17.14 (SD = 3.73) for the control stimuli.

As seen in Table 1, a 2 x 6 ANOVA (Gender x Color) was computed and revealed no main effects and no interactions to be significant. Additionally, post-hoc t-tests were performed to check for significance between the color which was, on average, recalled the most often and the color recalled the least often, see Figures 3 and 4. For men, the average number of green stimuli recalled (M = 18.93, SD = 2.25) was compared to the average number of yellow stimuli recalled (M = 15.40, SD = 3.92), this result was not significant, t (10) = 0.19, $p > 0.05$ (two-tailed). For women, the average number of red stimuli recalled (M = 20.14, SD = 2.64) was compared to the average number of black stimuli recalled (M = 17.14, SD = 3.73), this result was not significant either, t (13) = 0.11, $p > 0.05$ (two-tailed).

Discussion

The results did not significantly validate the hypothesis. Regardless of gender, color had little to no effect on the participant's short-term memory for this particular task. However, since each participant saw the stimuli in only one color, this study did not account for differences in attention due to coloration of the stimuli. Brandt (1943) found evidence that a person's attentional value may fluctuate due to coloration of stimuli. In addition, Camgöz, Yener, and Güvenç (2004) found that colors which were bright and highly saturated attracted more attention than those which were less bright and not as highly saturated. Thus, if the participants would have seen the stimuli in multiple colors, their attention may have fluctuated between each of the colors, which may in turn influence the results.

There was also a difference between the average number of non-emotion based words, nonsense syllables, and numbered stimuli recalled in this study. Participants recalled numbers and non-emotion based words much more easily than they recalled nonsense syllables. A possible reason for this effect may be due to the common use of words and numbers in daily life, whereas nonsense syllables are not as frequently used. Another possible reason for this effect could be due to the limited capacity of short-term

memory. Miller (1956) stated that the capacity of short-term memory is anywhere from five to nine items. Since nonsense syllables are more difficult to chunk together than words and numbers, they would occupy more of the limited storage of the short-term memory.

In addition, D'Argembeau and Van der Linden (2004) found emotionally-based-colored words were recalled significantly more often than were non-emotion-based-colored words. Furthermore, using procedures similar to those in this current study, Sleeth (2004) used pictorial stimuli instead of verbal stimuli to find significance between color and memory. On the other hand, this current study found little to no evidence that coloration has an effect on verbal stimuli in short-term memory, but according to Morales (2003), color is more likely to manipulate long-term memory than short-term memory. Thus, different designs have produced varying results; this study did not find colored verbal stimuli to have a significant influence on short-term memory, but this study does not prove that color does not have any effect on memory.

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Source	SS	df	Mean Square	F
Gender	12.55	1	12.55	1.25
Color	20.80	5	4.16	0.42
Gender*Color	51.18	5	10.24	1.02
Error	721.79	72	10.03	
Total	27058.50	84		

Table 1. Analysis of Variance for Gender and Recall of Colored Stimuli

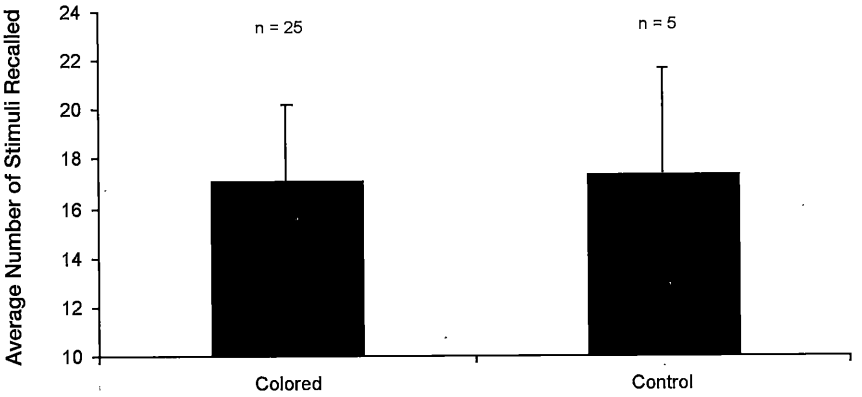

Figure 1. Average number of correctly recalled stimuli with standard deviations by men for colored and control stimuli.

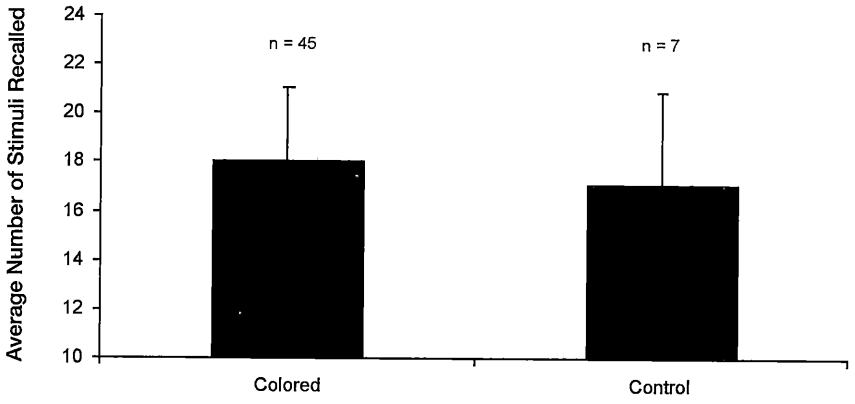

Figure 2. Average number of correctly recalled stimuli with standard deviations by women for colored and control stimuli.

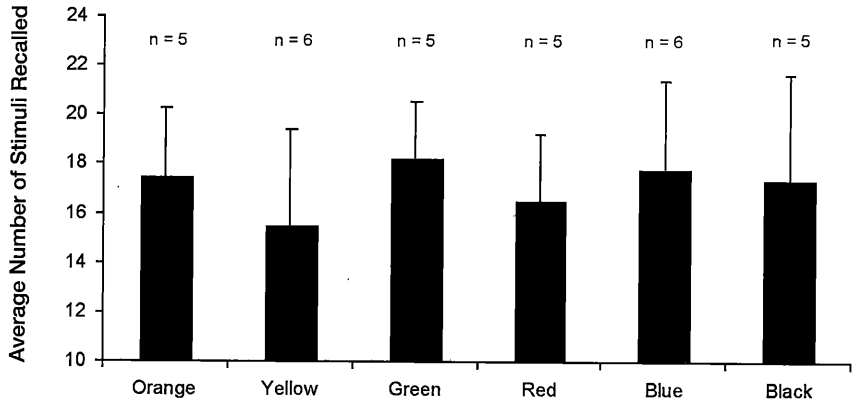

Figure 3. Average number of correctly recalled stimuli with standard deviations by men for each colored and control stimulus.

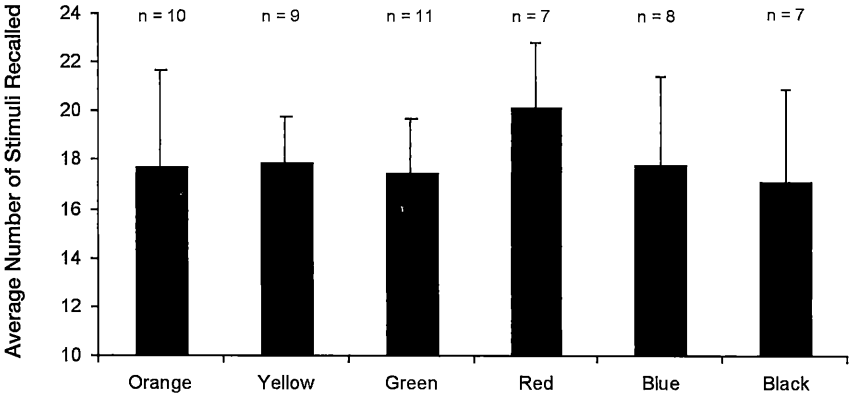

Figure 4. Average number of correctly recalled stimuli with standard deviations by women for each colored and control stimulus.

The Effects of Perceived Authority on Suggestibility in Interrogation-like Situations: A Pilot Study

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ABSTRACT

The effect of perceived authority on suggestibility was assessed. Two groups of participants were used, one receiving perceived authority and one receiving none. A military student dressed in fatigues administered the procedure to the first group and a civilian student in street clothes administered to the second. The Gudjonsson Suggestibility Scale was used to assess the suggestibility of each group. No significant difference between the groups was found.

STUDY

Memory is studied for many reasons, one being to discover the reliability of human memory. The study of memory reliability is of special interest to the criminal justice system. Findings in this area have a great impact on how eyewitness testimony is collected and interpreted.

In one recent study, participants were "tricked" into believing they had met Bugs Bunny, a Warner Brothers character, during a childhood visit to Disneyland (Loftus, 2004). The experimental group viewed an ad in which Bugs was seen next to the Disney Castle after which 16% of them said they had met Bugs at Disneyland, and later studies showed that with multiple exposures to the misleading ad the percentages of this occurrence rose (Loftus, 2004). This study demonstrates the malleability of memory. Clearly it is impossible to meet a Warner Brothers character at the Disneyland Park, but this impossibility had no effect on those that recalled meeting Bugs at Disneyland. Furthermore, in a follow up study participants were asked to rate the degree to which certain cartoon characters were associated, for example: Mickey and Minnie Mouse or Mickey Mouse and Bugs Bunny. Those who had fell for the fake Disney ad in the earlier study rated Bugs Bunny as more highly associated with various other Disney characters than did those who were not exposed to the misleading ad. These results suggest that the thought process of the people exposed to the fake ad had been affected (Loftus, 2004). It appears that an individual can not tell the difference between a memory that is false and one that is real. Loftus discusses precisely that.

"Psychological studies have shown that it is virtually impossible to tell the difference between a real memory and one that is the product of imagination or some other process" (Loftus, 2002). Loftus says that memories are susceptible to "post-event information" which can alter what individuals believe they experienced. This can happen in everyday situations, but it becomes especially troublesome when it happens with a criminal situation. People who have been witnesses to crime can be affected by many forms of "post-event information." These forms include: talking with others about the crime, being exposed to media coverage about the crime, and being asked leading questions. This "post-event information" can do more than change a detail here and there; it can create an entirely false memory for the event (Loftus, 2002).

Nourkova, Bernstein and Loftus (2004) looked at how effectively traumatic memories from the past could be altered. The study included 80 Russian participants who had memories of two terrorist bombings in Russia. The investigators tried to convince these participants that they had seen wounded animals in the media coverage of the bombings. A minority of the participants were convinced that they had seen a wounded animal in media coverage of one of the bombings. This study shows that even memories which have strong emotion attached to them can be altered (Nourkova, Bernstein & Loftus, 2004). Still some may argue that these results have no significance because the participants are not in a situation where the recollection of their memory is going to have dramatic effects such as in a criminal investigation. These critics argue that in order to create realistic results the participant must believe that their memories are going to be used in a criminal trial and that they are speaking to an actual law enforcement official.

Before the creation of Human Subjects Committees which protect human rights in research settings, Bernheim conducted a study which may be the first instance of implanted memory documented. Rosen, Sageman and Loftus (2004) discuss this study in an article. Bernheim described to one of his patients, Marie, the rape of a female child. He described the rape in great detail and repeatedly told Marie that she had witnessed it. Three days after this Bernheim had a lawyer friend of his come and talk to Marie and she told him the story in detail and said that she would testify to it and "was ready to swear before God and man" (Rosen, Sageman & Loftus, 2004). This case of implanted false memory certainly shows that it is possible for the human memory to be reshaped to the point that the individual would believe false information to be fact, even in court to the detriment of an innocent person's freedom, or even life.

It is estimated that in 1999 about 7,500 people have been wrongfully convicted of serious crimes in the United States due to mistaken memories (Loftus, 2003b). One example of this is the case of Ronald Cotton, who was wrongfully convicted of rape in 1986. The victim identified Cotton as her rapist, but he was later exonerated. In response to cases like this one Loftus (2003a) suggests a new oath: "Do you swear to tell the truth, the whole truth, or whatever it is you think you remember?" Perhaps more precise procedures for collecting eyewitness accounts and identifications would help to cut back on these miscarriages of justice. "Research has revealed the limits of human memory; now the courts need to incorporate these findings into their procedures" (Loftus, 2002).

Eyewitness memory errors account for more wrongful convictions than all the other causes combined (Wells & Loftus, 2003). One step towards proper procedures for collection of memory evidence has been taken. The United States Department of Justice came up with a set of national guidelines for collecting eyewitness evidence which include such recommendations as: asking open-ended questions and avoiding leading questions, and not interrupting the witness during interrogation (Loftus, 2002). These guidelines are a step in the right direction and as long as police officials take these recommendations seriously, there should be some improvement in this area.

The current study takes a look at memory in an interrogation-like situation and measures the suggestibility of participants who are exposed to interviewers with different levels of perceived authority. Suggestibility is defined by Gudjonsson and Sigurdsson (2004) as "the susceptibility of people to give into leading questions and interrogative pressure." Suggestibility was measured using the Gudjonsson Suggestibility Scale (GSS) which measures three things: yield to leading questions in two administrations of twenty questions (yield 1 and yield 2), one before and one after negative feedback; shift in answers from the first administration of the questions to the second; and total suggestibility which is yield 1 and shift added together (Gudjonsson & Sigurdsson, 2004). It is predicted that increased perceived authority will produce increased suggestibility when compared to a situation with little or no perceived authority.

METHOD

Participants

Nineteen female undergraduates participated in the study; ten in the experimental group and nine in the control. Random assignment was used. The participants were recruited from introductory level psychology classes at South Dakota State University. For participating in the study, participants were given extra credit points in their psychology class. Both investigators completed the NIH online training and the American Psychological Association's Ethical Guidelines were followed.

Materials

During the procedure, the investigators used a cassette player and cassette recording of the story to present the story to participants. A mini disk recorder was used to record the sessions so that the participants' answers could later be transcribed verbatim. Pencil, paper, and a clipboard were used by the investigators during the questioning to give the impression that the answers were being recorded as they were given. The Gudjonsson Suggestibility Scale (GSS) was used to measure suggestibility of the participants.

Procedure

Individually, each participant was given an opportunity to read and ask questions about the implied consent form, and was then asked to enter the procedure room where the interviewer was waiting. The interviewer was different depending on the group the participant was assigned to. The control group participants were exposed to a female

interviewer in street clothes who demonstrated little authority over the participants, while the experimental participants were exposed to a female interviewer dressed in army fatigues who demonstrated a higher level of authority over the participants. The participant was greeted by the interviewer and then asked to answer some questions to access her memory. After answering the questions the participant was asked to listen to a story on tape and was instructed to pay close attention because she would be asked to recall the information. The story on tape was played, after which the participant was asked to repeat everything she could remember. Twenty questions about the story were then asked and the participant was told to answer to the best of her ability. After all twenty questions were answered the interviewer indicated to the participant that she had made a number of errors and therefore must answer the questions again. The participant was also told to be more accurate the second time and the questions were asked again. After completion of the second round of questions the interviewer left the room and one of the investigators entered to debrief the participant.

Results

The participants in the control group (no perceived authority) showed a moderate level of suggestibility (M= 12.44, SD= 5.83). The experimental group also demonstrated a moderate level of suggestibility (M= 11, SD= 6.18) with the mean slightly lower than that of the control group. This relationship can be seen in Figure 1. A one-tailed t-test showed no significance, t (17) = -0.52, p = .30 (one-tailed).

Discussion

The participants in both groups had similar scores, with the control group scoring slightly higher on the suggestibility scale than the experimental group. There are several variables that could have caused these results. One is the limited number of participants in the study. Another is the difference in interviewers; although they were both female and around the same age, their differing personalities could have affected the results. The participants in the control group could have identified with their interviewer and therefore tried harder to please her by giving "more accurate" answers to the questions during the procedure; whereas the experimental participants may have felt offended by their interviewer's unfriendly demeanor and therefore refused to "be more accurate" for her.

In opposition to this theory, the interviewers could have failed to produce a difference between their demeanors. Bain, Baxter & Fellowes (2004) looked at suggestibility as a function of demeanor of the interviewer, and the interviewers' ability to vary their demeanors. The participants rated their interviewer and these results showed that the "friendly" interviewer was rated as friendly, and respectful, among other traits, and the "abrupt" interviewer was rated as firm, stern, and authoritative, among other traits. In this study, participants in the friendly group scored significantly lower on suggestibility than those in the abrupt group. These results support the current hypothesis and suggest that perhaps there was not sufficient difference between the demeanors of the two interviewers.

An additional variable is if each participant was familiar with the military, since the authoritative interviewer was dressed in military fatigues. If participants were familiar with the military, her attire may not have been affective in giving off the impression of authority. There were three participants in the control group with familiarity with the military and six in the experimental. It is very possible that these six participants did not yield to the interviewer's authority because they did not feel threatened by it. Although in the current study the results do not support the hypothesis, it is possible that if the variables described above were controlled in another study, supportive results could be found.

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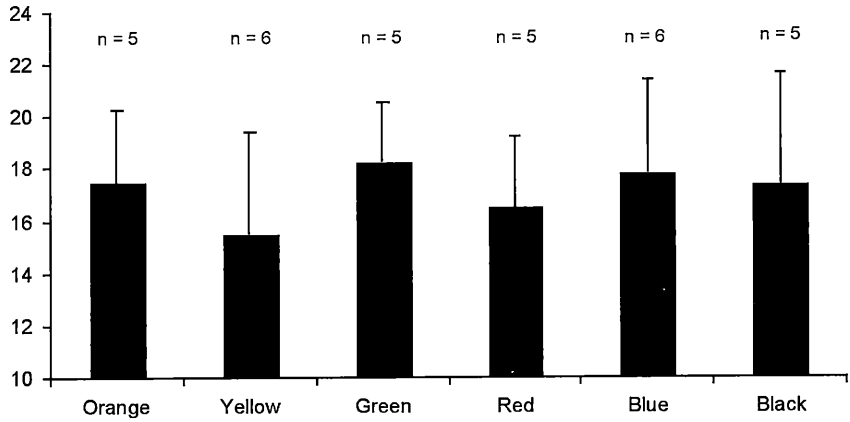

Figure 1. Means and standard deviations of the control and experimental groups.

Optimal Spacings for Two Common Landscape Irrigation Sprinklers

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Faculty Advisor: Dr. Todd P. Trooien
Department: Agricultural and Biosystems Engineering

ABSTRACT

A simple irrigation system providing sufficient watering needed to make a plant grow is not very difficult. However, to set up a system which provides uniform distribution of water is more difficult. Uniformity coefficients such as Distribution Uniformity and Christiansen coefficient of Uniformity can be used to compare systems and layouts. After measuring the distribution of water from a single sprinkler, a densogram is created with software. The densogram is a numerical and graphical representation of the overlap pattern that the sprinklers create on a given area of surface that is being irrigated. By varying the distance between the sprinklers and also changing the pattern in which they are laid out, the program is able to show which layout is the most efficient. Optimum sprinkler spacings (resulting in the greatest uniformity) for a commonly-used rotary sprinkler were 38 feet for a triangular layout and 31 feet for a square layout. For the square layout, there was little loss of uniformity for spacings up to about 48 feet. For a commonly-used spray sprinkler, optimum spacings were 12 feet for a triangular layout and 15 feet for a square layout.

INTRODUCTION

Many people have the misconception that water sprinkler systems are to be set up so that the radius of ground covered by each sprinkler is to just barely reach the coverage of another sprinkler. However; sprinklers should be designed to be set up so that the overlap of adjacent sprinklers creates the most uniform water application pattern.

As early as about 5000 BC the Egyptians along the Nile River used irrigation systems (Hoffman et al., 1990). Modern systems are becoming more advanced by each passing day. But the goal of all irrigation systems remains the same: apply water uniformly, thereby minimizing both over-watered and under-watered areas. The one-dimensional uniformities of multiple sprinklers using various spacings and layout geometries are able to show how water may be distributed along a single path. This information is not enough to get a thorough understanding of what types of patterns and how the grid of a system and layout of sprinklers will appear. A better understanding can be gained by calculating two-dimensional uniformities based on multiple sprinklers, varying their layout and spacing. By testing all practical combinations of layout and spacing, optimal system designs can be identified. The objective of this study was to

determine the optimum sprinkler spacings for two commonly used landscape irrigation sprinklers.

MATERIALS AND METHODS

The two sprinklers used for this experiment were the TORO 800 Rotor and the TORO 570 Spray. These sprinklers are common in landscape irrigation systems. A sprinkler test stand was used for the previous experiment to collect the data. Our sprinkler test stand was a barrel with a small opening that restricted water application to about 15° of the entire horizontal arc of coverage. A water pressure gauge was attached to the hose running from the faucet to the sprinkler inside of the barrel. The water pressure at the sprinkler was maintained at 30 psi by adjusting a gate valve. Water was collected using plastic cones placed in one foot increments. Volume caught in each cone was divided by the test time then converted to calculate the precipitation rate in inches per hour. Two tests of precipitation rate were performed. The results of the two tests were similar and the averages of the two tests are used in this study.

Uniformity of application was calculated using the precipitation rate measurements and the SPACE PRO program (Center for Irrigation Technology, 2000). Using the previously recorded precipitation rate data, Distribution Uniformity (DU) and Christiansen coefficient of Uniformity (CU) percentages are calculated. In order to fully understand the way that the DU and CU are changed according to the distances that the sprinklers are from one another, one must first realize how they are calculated. The DU is calculated as (Rochester, 1995):

$$DU = 100\left(\frac{X_{LQ}}{X}\right) \tag{1}$$

where: X_{LQ} is the average of the lowest 25% of the precipitation rates and
X is the mean of all precipitation rates

The CU is a little more difficult. An advantage to using CU instead of DU is that it will give a more overall average instead of using the lower quarter of findings. The CU is calculated as:

$$DU = 100\left(\frac{X_{LQ}}{X}\right) \tag{2}$$

where: X_i is the i^{th} individual measurement of precipitation rate
X is the mean of all precipitation rates, and
n is the number of measurements

The initial step was to add a new profile record according to the type of sprinkler that is being used, and a graph of the data is then given. After the new profile is saved, a densogram was created and uniformities calculated. The program gives the choice of what type of layout the sprinklers are to be placed in, the water flow, and distance between the sprinklers. For this experiment, both the equilateral triangular and square sprinkler layouts were studied. The only factor that was varied is the distance between the sprinklers. Spacings were varied in 1-foot increments from 11 to 22 feet for the spray sprinkler and from 11 to 55 feet for the rotary sprinkler.

RESULTS: WATER DISTRIBUTION OF A SINGLE SPRINKLER

During the initial experiments of collecting the data along a straight line out from a single sprinkler, water application varied linearly with distance from the 570, a spray sprinkler (Figure 1). A linear equation can be fit to the water distribution from the spray sprinkler: PR = 32.5 - 2.5 * (feet from sprinkler). This equation can be used in a spreadsheet or other analytical tool to calculate water application at any point within an irrigation system. Calculating overlapping patterns is straight forward for linear water distributions such as the 570 but is more difficult for non-linear distribution. Water distribution from the rotor (800) was not linear (Figure 2). Other software tools such as Space Pro make such analysis more convenient.

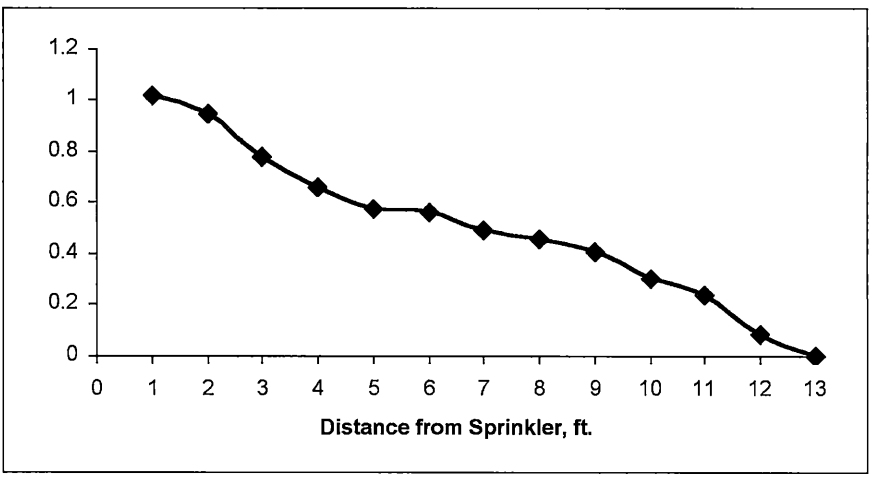

Figure 1. Precipitation rate with distance for the Toro 570.

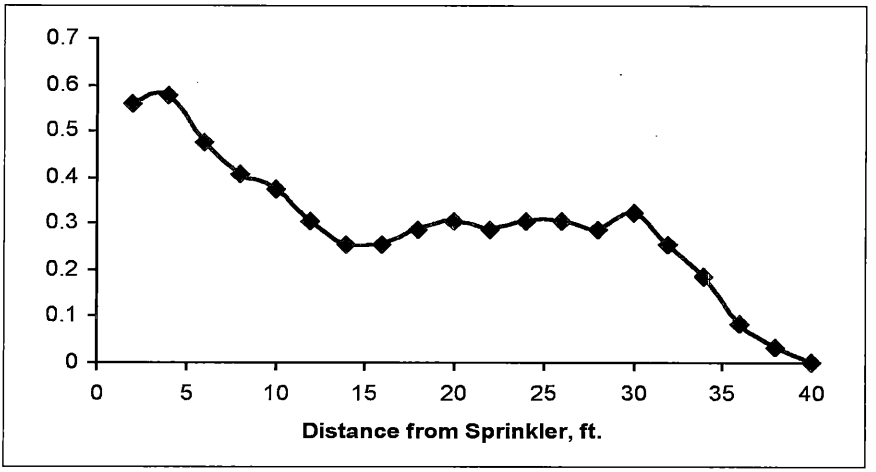

Figure 2. Precipitation rate with distance for the Toro 800.

RESULTS: WATER DISTRIBUTION OF SPRINKLER SYSTEMS

The optimal spacing (resulting in the greatest uniformity) for the Toro 800 rotary sprinkler in a triangular layout was 38 (Figure 3). This distance corresponds to the distance of throw of a single sprinkler (Figure 2). The DU and CU were 92% and 95%, respectively, with the sprinklers at this spacing. Experiment results show that when the distance between sprinklers is increased the DU and CU decrease. Likewise, when the distance is decreased between sprinklers, the CU and DU decrease because the center of the area receives excess water and others remain at the small accumulations of precipitation.

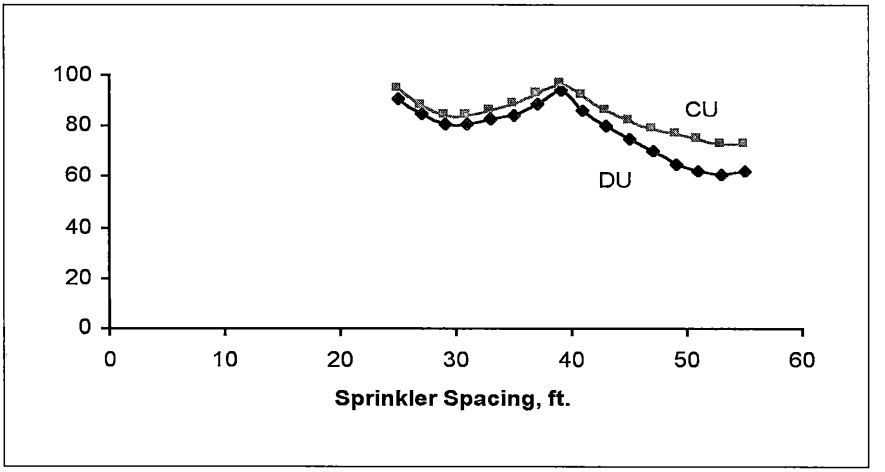

Figure 3. DU and CU of Toro 800 in a triangular layout.

When the Toro 800 Rotor sprinkler is in a square layout (Figure 4), the results vary slightly compared to the triangle layout. In this layout, when the distance between the sprinklers is extended slightly beyond 38 feet, the DU and CU increase slightly. These results are due to that when the distance is 32 feet, the precipitation at the center of the region is rather high even though it brings about the highest DU and CU. Separating the sprinklers helps and it was found that moving the sprinklers 45 feet apart brings the best DU and CU. At a spacing of 45 feet, the DU is 78% and the CU is 86%. Anything beyond that reduces the uniformity. Also different with the square layout compared to the triangle is that when the distance between the sprinklers is decreased, the DU and CU continue to increase because the overlapping of the sprinkler radii results in equal amount of water being fed to each part of the region.

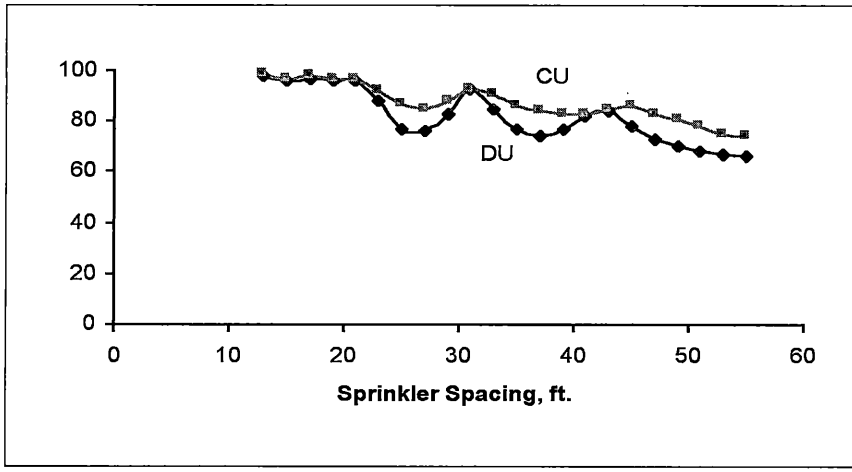

Figure 4. DU and CU of Toro 800 in a square layout.

When the Toro 570 Spray is in a triangular layout, the optimal spacing is 13 feet (Figure 5). When the distance between each sprinkler is 13 feet, the DU and CU are 95 and 97%, respectively. Using this layout offers a large amount of room for error in the distances between each sprinkler because from spacings 10 to 20 feet, the uniformity decreases only slightly. However, when the distance is increased or decreased beyond either of these points, the DU and CU decrease in percentages because of gaps and increases in water precipitation created all over the region being watered.

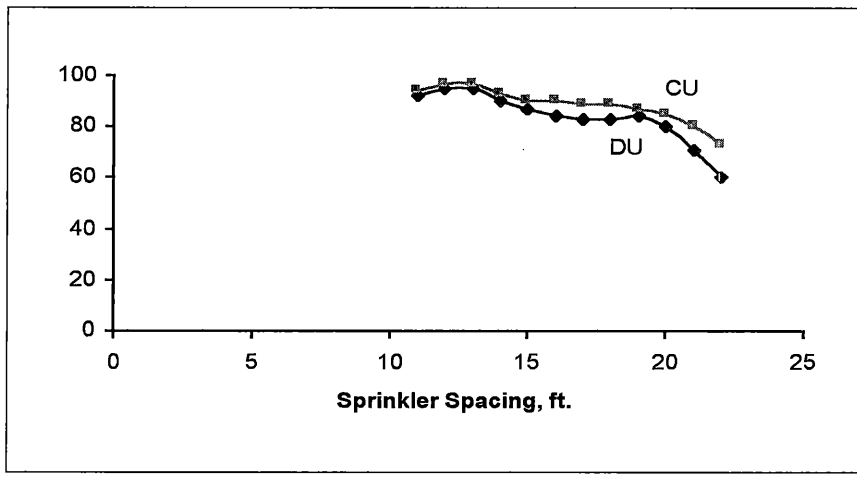

Figure 5. DU and CU of Toro 570 in a triangular layout.

When using the Toro 570 Spray in the program as a square layout, the most uniform spacing was 15 feet, giving a DU of 92% and a CU of 95% (Figure 6). When the spacing is increased, the DU and CU decrease dramatically (Figure 6). Likewise, when the distance is decreased between each sprinkler, the DU and CU is decreased because there are excess amounts of water near the bases of each sprinkler and gaps in the region surrounding the large amounts in the center.

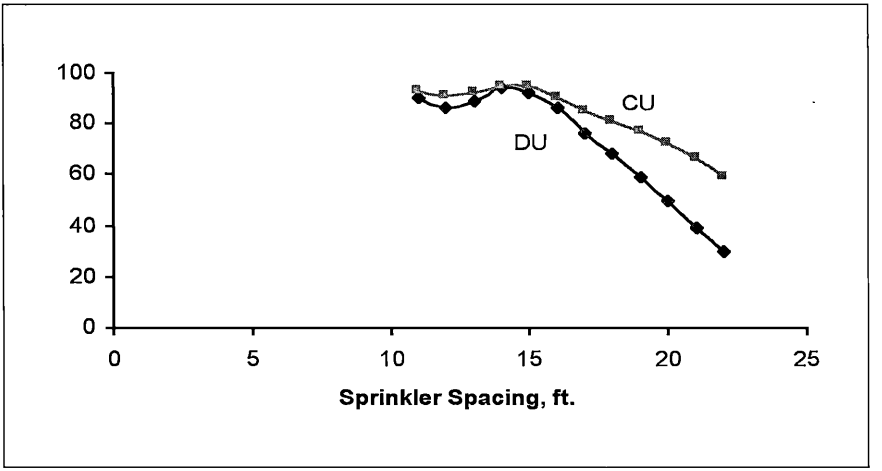

Figure 6. DU and CU of Toro 570 in a square layout.

Either of these sprinklers can be used to design an irrigation system that applies water uniformly. The maximum DU and CU values greater than 90% indicate that water is being applied uniformly. When local and installation conditions allow these sprinkler spacings, uniform water application can be achieved.

CONCLUSIONS

When using the Toro 800 Rotor in a square layout, setting the sprinklers 31 feet apart will create the most efficient distribution with a DU of 93%. When in a triangular layout, the optimal distance between the sprinklers is 38 feet apart giving a DU of 92%. The optimal spacing for the Toro 570 Spray in a square layout is 15 feet with a DU of 89%. For the 570 in a triangular layout, the optimal spacing is 12 feet, giving a DU of 95%. The variation of uniformity due to spacing change was small for the triangular layout, resulting in uniform water application at a variety of spacings.

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Determination of Trifluoroacetate by Ion Chromatography for Snow and Ice Analysis

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Department: Chemistry and Biochemistry

ABSTRACT

Trifluoroacetic acid (TFA) is a product of the atmospheric degradation of hydrochlorofluorocarbons (HCFCs) and hydrofluorocarbons (HFCs). HCFCs and HFCs are widely used substitutes for chlorofluorocarbons (CFCs) that have been found to contribute to the loss of stratospheric ozone. Deposits of TFA with precipitation (rain and snow) can lead to its presence and accumulation in the aquatic and cryosphere environments, with unknown long-term environmental consequences. Investigation on TFA impact and its fate in the environment requires sensitive, accurate, and fast analytical methods. The determination of trace concentrations of TFA using the technique of ion chromatography (IC) is investigated in this study, with a potential application of the technique for TFA in Antarctic snow samples. It was found that the IC detection and quantification of TFA as an anion is free from chromatographic interference by major anions in Antarctic snow. The detection limit using a procedure without preconcentration was found to be 0.2 ppb TFA, while the limit of quantification is as low as 0.5 ppb. Keywords: trifluoroacetic acid, trifluoroacetate, ion chromatography, snow, ice.

INTRODUCTION

The stratosphere is a part of the atmosphere at the altitude range of approximately 12 to 50 km. The stratosphere contains a relatively large amount of ozone (O_3) that absorbs short-wavelength ultra-violet (UV-B) radiation from the sun. The high-energy UV-B photons, if allowed to reach Earth's surface, can damage biological molecules and cells in plants and animals (1). Therefore the stratospheric ozone layer provides important protection for life on Earth.

The emission and atmospheric accumulation of chlorofluorocarbons (CFCs), a class of compounds commonly used as propellants in aerosols and refrigeration gases prior to the 1990s, has been found to cause severe losses of stratospheric O_3, especially O_3 over Antarctica during the austral spring (i.e., the Antarctica ozone hole). CFC molecules are chemically inert under ground level atmospheric conditions, a property that made CFCs popular in commercial products that required the use of stable gases. Due to the inert

chemistry of the CFC molecules, once in the atmosphere, they are able to resist chemical reactions that would normally remove pollutants from the atmosphere. The stability of these molecules results in long atmospheric residence times and allows significant amounts to drift into the stratosphere. The high-energy solar radiation in the stratosphere breaks down the CFC molecule and releases chlorine radicals that serve as a catalyst in reactions that destroy O_3 molecules. As a result of the chlorine-catalyzed destruction of ozone, a severe loss of the ozone layer has been observed over the poles of the earth.

Efforts to stop the destruction of the ozone layer have led to a ban on CFCs and the introduction of substitutes such as hydrochlorofluorocarbons (HCFCs) and hydrofluorocarbons (HFCs). HCFCs and HFCs were designed to break down in the troposphere (0-12 km), so that they do not pose a threat to the stratosphere.

The long-term effect of HCFCs and HFCs on the environment as a result of their degradation is relatively unknown. One of the products from the degradation of HCFCs and HFCs, trifluoroacetic acid (TFA), is of particular concern because it tends to accumulate in the environment. The majority of TFA found in the environment is formed as a product of the atmospheric degradation of HCFCs and HFCs (2).

The CFC substitutes, HCFC-134a, HCFC-123 and HCFC-124, are considered to be the primary source of TFA. One of the products from the initial degradation of these compounds is trifluoroacetyl fluoride (CF_3COF) which is taken up by clouds, subsequently hydrolyzed to TFA (3) and deposited on Earth's surface through precipitation. TFA is a very persistent chemical in aqueous environment and may have the potential to accumulate in lakes, rivers, and in glaciers and ice sheets (4). The accumulation of TFA in these ecosystems is an environmental concern because of the unknown long-term consequences. The amount of TFA occurring naturally in the environment is known to be very small so most TFA found in the environment is likely to be man-made. There is evidence suggesting that >95% of TFA found in surface water comes from precipitation (4).

TFA concentration in precipitation was found to be very low (5). However, TFA concentrations in surface water in Europe were found to be significantly higher (4). This may indicate that surface waters could be accumulating TFA and other degradation products of air pollutants such as HCFCs and HFCs. Industrial operations are thought to be another source of environmental TFA, because high concentration levels were reported in river waters near industrialized areas (2).

The current method for the determination of TFA in precipitation and surface waters relies on the technique of gas chromatography and mass spectrometry (GC-MS). GC-MS requires a water soluble analyte to be converted to a volatile compound, usually through derivatization, followed by extraction using an organic solvent prior to quantitative determination by GC-MS (2). The technique is usually very sensitive but requires a complex sample preparation process that results in long analysis time. The typical efficiency of TFA extraction for GC-MS determination is found to be 92 ±3% (2).

The objective of this work is to determine whether TFA can be measured using an alternative technique, ion chromatography (IC) and to investigate if IC would be suitable to measure TFA in Antarctic snow and ice core samples where the concentration of TFA is believed to be very low. Since only ionic analytes are detectable in IC, TFA must be a

relatively strong acid (i.e., with a large acid dissociation constant, K_a), in order to be detected by IC. The K_a of trichloroacetic acid, a structural analog of TFA, is $3.2x10^{-1}$. Since TFA is a stronger acid than trichloroacetic acid because of the higher electronegtivity of fluorine than chlorine, this suggests that TFA is nearly a strong acid and would be expected to completely ionize in aqueous solutions to TFA^- (6).

Ion chromatography is well suited to the analytical measurement of ionic analytes in aqueous solutions at sub-ppb concentrations without preconcentration. Using ion chromatography for TFA determination would allow for very minimal sample preparation and consequently shorten the analysis time of GC-MS. This technique has been used for the detection of low concentrations of commonly found anions (Cl^-, NO_3^-, SO_4^{2-}, formate and acetate) in Antarctic snow and ice core samples.

This study is aimed at determining the ability of IC to quantitatively measure TFA, with the ultimate goal of applying this technique to the determination of low concentrations of TFA in Antarctic snow and ice core samples. The analysis of Antarctic snow and ice samples for TFA may yield insight into the annual amount of TFA being deposited on the Earth's surface and a timeline of the trend in TFA deposition.

METHODS

A Dionex (Sunnyvale, California) DX600 ion chromatograph with an AS11 column with suppressed conductivity detection was used for the analysis of TFA^- and other common anions. A Dionex AS40 autosampler with 5 mL plastic vials was used to hold samples and transfer them to a 500μL sample injection loop. The relatively large 500μL injection volume was used to obtain sufficiently large peaks for very low (a few ppb) concentrations of TFA^- and other common anions. A dilute solution of NaOH was used as the eluent at a flow rate of 1.00 mL/min for the isocratic elution of the anions. The eluent and all calibration standards were prepared using deionized water with resistivity > 18 MΩ cm at 25° C. The concentrations of anions measured in this study are very low and susceptible to contamination. As a precaution against contamination, all glassware and other containers were thoroughly cleaned before being used.

Standard solutions of TFA were prepared from concentrated (99%) TFA, supplied by Aldrich Chemicals. An initial solution of 1000 ppm TFA was made by volumetrically diluting the concentrated TFA and then storing in 100 mL plastic containers that had been thoroughly washed with deionized water. Further dilutions were made from this stock solution to prepare test samples and calibration standards. For example, a test solution of 250 ppb TFA was prepared to determine and optimize the retention time of TFA^- vs. other common anions. A typical calibration curve for TFA was created using a blank and four standards at 0.5, 1.5, 2.5, and 5 ppb. All calibration standards were freshly prepared for each day of analysis. The common anions that could interfere with the TFA^- peak in a chromatogram include F^-, Cl^-, NO_3^- and SO_4^{2-}. Standard 1000 ppm solutions of these anions were prepared and stored in 1000 mL plastic containers. Solutions of individual anions and a mixed standard containing F^-, Cl^-, NO_3^- and SO_4^{2-} were analyzed separately to determine the retention time of each anion. The strength of the eluent or

NaOH concentration was then adjusted to optimize chromatographic separation of the anions and to avoid any overlap between peaks of the four anions and the TFA peak.

To investigate a pre-concentration technique to lower the TFA detection limit, standard solutions of 0.1 to 1.0 ppb TFA^- were prepared in clean 1 L volumetric flasks and transferred to clean 1 L glass beakers. The solutions were then heated on a hot plate and allowed to evaporate without boiling. The evaporation was done in a Laminar flow hood to ensure that the laboratory air would not contaminate the solutions. The volume of each solution was first reduced to about 25 mL and then transferred quantitatively into a clean 50 mL beaker which was heated again to reduce the volume to less than 10 mL. At this point, the solution was immediately transferred to an autosampler vial and analyzed using the IC.

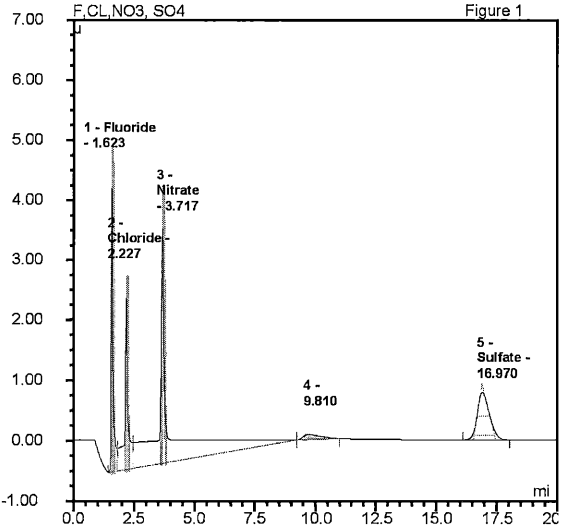


Figure 1. Chromatogram of 37.5 ppb F^- and Cl^- and 150 ppb NO_3^- and SO_4^{2-}.

RESULTS AND DISCUSSION

An initial eluent solution of 7 mM NaOH eluded the chloride on the shoulder of the negative peak (water dip), making it difficult to accurately measure the concentration of chloride. The eluent strength was gradually weakened to delay the elution of chloride until its retention time was approximately 3 minutes, where it would be separated from the water dip. This occurred when the eluent strength was about 3 mM. The retention time of TFA^- was found to be in the range of 3.9 –4.1 minutes using a 3 mM NaOH eluent.

The retention times of common anions found in snow and ice samples were determined to ensure no peak overlap would occur involving the TFA^- peak. Peak overlap must be prevented so that each peak represents only one anion and therefore can be measured separately and quantitatively. Figure 1 shows a chromatogram of a test solution to find the retention times of F^-, Cl^-, NO_3^-, and SO_4^{-2} and to determine the proximity of their peaks to each other and the TFA^- peak shown in Figure 2 using the 3 mM NaOH eluent. The F^- and SO_4^{-2} peaks elude before the Cl^- peak and after the NO_3^- peak respectively and therefore posed no threat to overlap with the TFA^- peak.

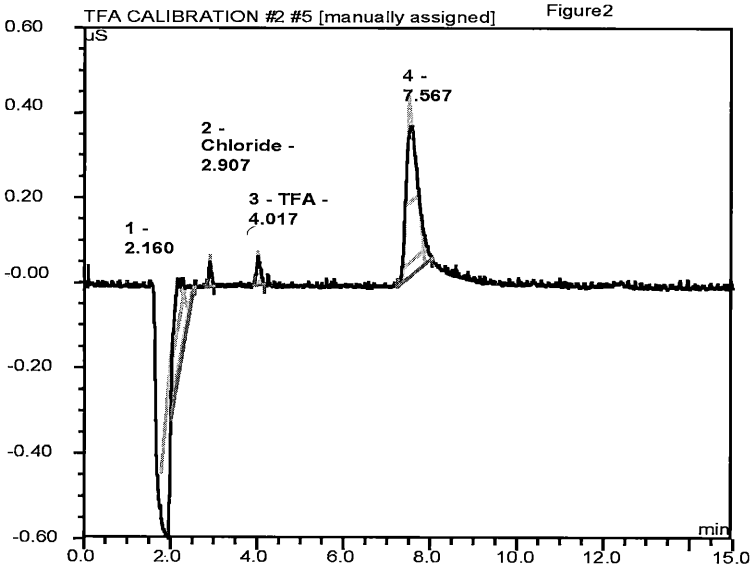


Figure 2. Chromatogram of 6 ppb TFA^- with retention time of 4.017 minutes.

The retention time for TFA^- is shown in Figure 2. The nitrate and chloride peaks were the most likely peaks from Figure 1 to overlap the TFA^- peak in Figure 2 due to the similar retention times. The chromatogram in Figure 3 shows NO_3^- and Cl^- concentrations of 200 ppb and 100 ppb respectively which are greater than the average NO_3^- and Cl^- concentrations of 100 and 30 ppb respectively, in Antarctic snow and ice samples (7,8). Figure 3 shows that even at extremely high NO_3^- and Cl^- concentrations the peaks do not overlap the TFA^- peak.

The TFA calibration curve shown in Figure 4 was used to determine the detection limit of the IC for TFA^-. The calibration curve is composed of TFA concentrations of 0.5, 1.5, 2.5, and 5 ppb and shows a correlation coefficient of 0.9998. The detection limit is defined as the TFA concentration that gives a peak height 3 times average noise level of the chromatogram and is the lowest detectable concentration of the analyte. The noise level is determined by taking an average of the heights of the random peaks in the baseline.

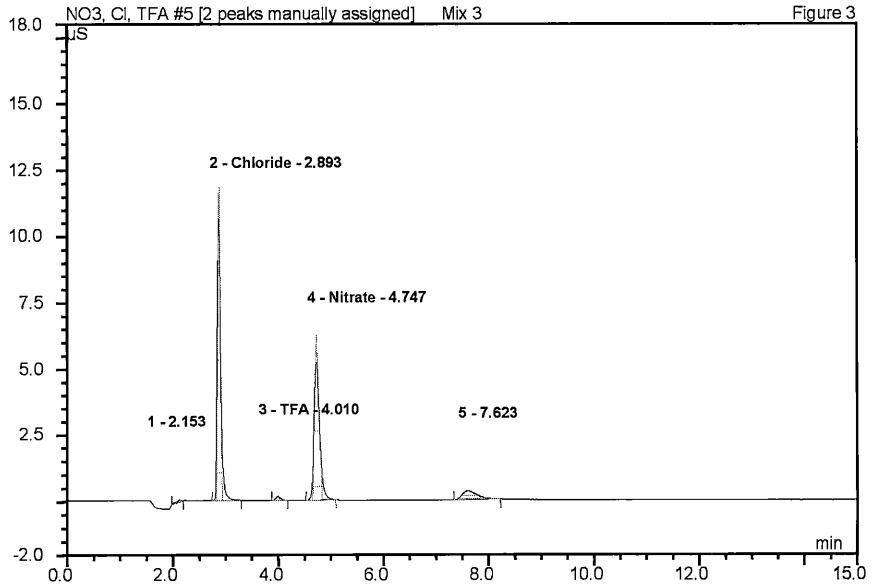
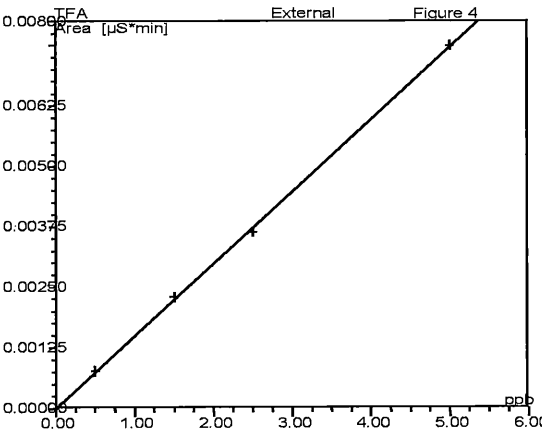


Figure 3. Chromatogram showing retention times of analytes in a solution of 100 ppb chloride, 10 ppb TFA^- and 200 ppb NO_3^-.

Using the above approach and an average baseline noise level of 0.001µS, the detection limit of IC for TFA^- was determined to be 0.2 ppb. The limit of quantification of IC for TFA^- was 0.5 ppb, which is the lowest concentration of TFA^- that can be accurately quantitatively measured.



The analysis of TFA by GC-MS on Antarctic snow by Von Sydow et al. detected a mean TFA concentration of 0.04 ppb or 40 ppt in layers that were formed in the 19th century (9). That same study found that the concentration of TFA in individual rainfalls over Mace Head, Ireland varied from 2 to 92 ppt.

Figure 4. TFA^- calibration curve of 0.5, 1.5, 2.5 and 5.0 ppb.

This detection limit of the IC method for TFA is not low enough to measure TFA in Antarctic snow and ice samples directly by ion chromatography because the expected TFA concentration is about 0.04 ppb (9).

The results from the pre-concentration investigation did not yield useful data. Due to high baseline noise resulting from the concentrated impurities in the evaporated solutions, it was impossible to quantify the TFA^- peak accurately. Estimates of the TFA^- peak concentration were much smaller than would be expected after concentrating the samples to one hundredth of the original volume. More work would be needed to find the cause of this problem or to determine whether the evaporation technique will be applicable to the TFA^- analysis on snow and ice samples.

CONCLUSION

The analysis of TFA through the use of ion chromatography found that it was possible to detect and accurately measure concentrations of TFA as low as 0.5 ppb with a detection limit of 0.2 ppb. Ion chromatography will offer less sample preparation and shorter analysis time than GC-MS. However, snow and ice samples will need to be pre-concentrated in order to measure the expected low concentrations of TFA. Ion chromatography would be a better technique (more accurate and faster) than GC-MS to measure TFA in surface waters and in snow and ice core samples where the concentrations of TFA are above 0.5 ppb.

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The Fate of 2,4-D in Intact Soybean (*Glycine max*)

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ABSTRACT

About 479,000 pounds of 2,4-D (2, 4-dichlorophenoxyacetic acid) was applied to South Dakota's crops in 2000. 2,4-D can injure or reduce yield of soybean (*Glycine max*) if deposited on growing plants. This study determined uptake, translocation, and metabolism of ring-labeled-^{14}C -2,4-D in soybean at the third trifoliate (V3) stage of growth. Plants were harvested and partitioned into four parts from 1 hr (HAT) to 10 d (DAT) after treatment. Thin layer chromatography techniques were used to determine if ^{14}C remaining in the tissue was parent chemical or metabolite. 2,4-D uptake ranged from 39% at 1 HAT to 74% 6 DAT. By 10 DAT, ^{14}C translocated to the youngest tissue (10%) and older tissue (8%). All ^{14}C recovered from the treated leaf 1 HAT was parent 2,4-D, however, at 24 HAT and later, only about 30% of the ^{14}C remained as 2,4-D whereas 70% of the ^{14}C was observed as a more water soluble compound.

INTRODUCTION

In South Dakota, 100% of corn acres, 98% of soybean acres, 93% of spring wheat acres and 56% of winter wheat acres were treated with herbicide in 2000 (USDA National Agricultural Statistics Service, 2001). 2,4-D (2, 4-dichlorophenoxyacetic acid) is a chlorinated phenoxy compound. 2,4-D is a plant growth regulator used worldwide to control broadleaf weeds. 2,4-D has been available since the mid-1940's and is applied in many situations today. About 479,000 pounds acid equivalent (a.e.) 2,4-D was applied to 56% of SD spring wheat acres, 26% of SD winter wheat acres and 2% of SD corn acres in 2000. Application rates of 2,4-D to corn, spring wheat, and winter wheat averaged about 0.95, 0.37, and 0.50 lb a.e. per acre, respectively (USDA National Agricultural Statistics Service, 2001). According to the 2,4-D label this product cannot be sprayed when the wheat crop is in the boot to dough stage. These figures do not include the additional pounds of 2,4-D used in pastures, rangeland, woodlands, or home usage.

2,4-D may be applied to land prepared for soybean crops, but only as a pre-emerge application for burn down of emerged broadleaf weeds. The rate that is used depends on the interval before soybean planting. A producer can apply up to 0.5 lb a.e. per acre seven days before planting soybeans, or a 1 lb a.e. per acre rate can be used if there is a 30 day interval before planting (2,4-D Label). 2,4-D has very little soil activity; therefore, when applied preplant it should not affect soybeans.

The main cause of the problem with 2,4-D on soybean is drift or volatilization of the chemical from target areas onto emerged soybean plants. The fact that so much of this chemical is used in South Dakota greatly increases the potential for drift to occur. Drift occurs when 2,4-D is applied to adjoining corn or wheat fields and chemical is carried to soybean fields by the wind. The average wind speed in South Dakota during May and June is 5 to 8.5 MPH (SDSU Electrical Engineering, 2001). Volatilization is the movement of chemical due to evaporation and redeposition occurs when 2,4-D is applied at high temperatures around 85° F (2,4-D, Pesticide Fact Sheet, 2005). The average temperature in Brookings, South Dakota in May and June is 70° F with actual day to day temperatures as high as 90° F SDSU(Climate, 2005). In 2002, 16 days in June recorded temperatures of 80° or higher, within the first month after soybean emergence (June 1) (SDSU, Climate, 2005). These temperatures increase the risk of volatilization at a time when soybean is very sensitive to 2,4-D injury.

When 2,4-D comes into contact with soybeans, it can be very detrimental to a soybean crop. Some symptoms of 2,4-D affected plants include stem twisting (epinasty), leaf discoloration (chlorosis), and eventually death (necrosis). Some of these symptoms can be seen in plants just hours after application (Kelley et al., 2002). In addition it takes very little 2,4-D to cause damage to soybeans, only 10% or 0.06 lb a.e. per acre of the 0.61 lb a.e. per acre labeled rate in corn has been shown to cause a 7% yield loss in soybeans (Andersen et al., 2004).

2,4-D is a post-emergence translocated herbicide. It enters the plant through the leaf, and stem tissue and is then translocated through the plant via the phloem. 2,4-D has been used for more than four decades, yet research detailing the metabolism of the herbicide in intact soybean is difficult to find. Soybean was the focus of this study due to the 4.2 million acres of soybean planted per year in South Dakota and their susceptibility to 2,4-D damage. Injury complaints on soybean and other broadleaf plants due to growth regulator compounds, such as 2,4-D and dicamba, comprised about 66% of the injury complaints seen by the pesticide testing facility at SDSU in 2004 (D. Matthees, personnel communication). In most cases, however, the samples sent in near the end of the season contain little if any 2,4-D, although injury symptoms were often rated as severe. The objectives of this study were twofold. The first was to determine how much chemical entered the plant in a given time period and translocation patterns within a plant that has been affected by a low rate of 2,4-D. The second objective was to determine if soybeans metabolized 2,4-D and if so, the timeframe of when this occurred and what metabolites were formed.

MATERIALS & METHODS

Soybean plants (variety Surge) were grown in the greenhouse until they reached the third trifoliate (V3) growth stage. These plants were treated with the methyl-ester formulations of 2,4-D at an equivalent rate of 0.112 kg ae/ha (0.1 lb ae/a). 2,4-D was mixed with water (1:748) and applied to the plants using a garden spray bottle with the nozzle set to a fine mist. Aluminum foil was cut to fit around the stem of the plant and

covered the soil. The plant was misted twice with the 2,4-D solution to cover the leaf surfaces. The aluminum foil was removed and the amount of herbicide solution per plant was determined.

Once the soybean plants had been treated, the leaves were allowed to air-dry. The middle leaflet of the second trifoliate leaf was marked for identification. The marked leaf was then treated with 10 ul of uniformly ring labeled ^{14}C-2,4-D with a radioactivity level of 2.2 X 10^6 DPM. The plants were harvested at 1 hour after treatment (HAT), 6 HAT, 48 HAT, 6 days after treatment (DAT) and 10 DAT. Each harvest time was replicated three times, and the experiment was replicated in time.

At harvest, the leaflet treated with ^{14}C-2,4-D was removed from the plant, and the surface was rinsed with 3 ml of methanol. The rinsate was collected and 100-ul aliquot was transferred into a scintillation vial. Two ml of scintillation cocktail (Ultima Gold, Packard Bioscience BV, Meriden CT) was added to the rinsate aliquot and the vial was shaken and placed in a scintillation counter (Packard Bioscience Company, Meriden CT) to determine the level of radioactivity on the leaflet surface. The maximum counting time was 10 min or until 2 sigma reached 0.05. The remaining rinsate was labeled and stored.

Once the treated leaflet had been rinsed, the leaflet was freeze dried in liquid nitrogen and weighed. The leaflet was then placed into a bag and stored at -18 C until further processing. The remainder of the plant was partitioned into 1) the remaining 2 leaflets of the trifoliate, 2) leaves and stem above the treated leaf, and 3) leaves and stem below the treated leaf and stored as previously described.

One plant from each treatment period was selected for further processing. Each plant part was freeze dried using liquid nitrogen and weighed. The leaves were placed into a mortar, more liquid nitrogen was added and the leaves were ground up using a pestle. Five ml of methanol was added to the finely ground leaf tissue to extract the remaining herbicide in the remaining 2 leaflets of the trifoliate and leaves and stems above the treated leaf. Ten ml of methanol was added to the leaves and stems below the treated leaf and ground. The liquids from the ground materials were decanted into vials. A 100-ul aliquot was placed in 2 ml of scintillation cocktail and radioactivity determined as described above.

A 50-ul aliquot of extract (in two 25-ul increments) from the ground plant tissue of the treated leaflet and remaining 2 leaflets of the third trifoliate were spotted onto thin layer chromatography (TLC) plates (Whatman Inc. Clinton NJ). The first 25- ul aliquot was pipetted onto the plate, allowed to dry for several hours and then the second 25-ul aliquot was applied. One ul of ^{14}C- 2,4-D standard, containing about 24,000 DPM, was placed on 1 or 2 lanes on the thin layer chromatography plates.

The entire extract from the 48 HAT, 6 DAT and 10 DAT ground plant tissues above the treated leaf was reduced to near dryness using a stream of warm air. An aliquot of methanol was added back to the near dry materials. A 25-ul aliquot of this concentrated extract was counted by scintillation counting techniques previously described. Then 50-ul aliquots of these extracts were spotted onto TLC places as previously described.

The chromatography plate was developed using a (42:4:8) benzene/acetic acid/methanol solution (Eastin, 1986). The solution traveled to a height of 15 cm. The plate was allowed to air dry in the fume hood for 20 minutes and was then placed into a 100°C oven for 4 minutes. The plates were placed against a phosphorescent screen for

1 to 4 days. The energy of ^{14}C decay resulted in dark spots on the screen. The screen was then placed in a counter that detected the darkened areas and was reported as a function of pixel density.

Pixel density of the ^{14}C-2,4-D standard was used to determine the Rf value of 2,4-D in this developing solution. The Rf value of the standard was determined using the equation: Rf = height of 2,4-D movement/height of solvent movement (Weete, 1986).

The Rf values of high pixel density areas that were found outside the standard 2,4-D spot were calculated and were considered to be 2,4-D metabolites (or breakdown products), since they were still radioactive but did not have the same characteristic movement of 2,4-D.

RESULTS AND DISCUSSION

Plant Injury, 2,4-D Uptake and Translocation

There was a large amount of variation in the amount of 2,4-D applied to each plant as seen in Table 1. The variation in the amount of 2,4-D applied to each treatment ranged from 1X (0.78 mg/plant) to 4X (3.22 mg/plant). Although the chemical amount applied was the least for the 6 and 10 day harvest times, the injury symptoms were more severe than plants treated and harvested at the earlier times (Table 2 and Figure 1). Symptoms of 2,4-D injury included stem curling, epinasty, upper leaf curling, leaf cupping, chlorosis, and, finally, death. This increase in symptom severity was also observed in a field study done by Andersen et al. (2004).

The amount of 2,4-D taken up over the 10-day experiment varied with the amount of time that the 2,4-D was left on the plant (Table 3). Total uptake ranged from 40% 1 HAT to 74% 10 DAT. The percent of radioactivity that entered the plant at 6, 24, and 48 HAT was about 55% of the total added. The percent of uptake at 6 and 10 DAT was about 70% of the amount added. The amount of ^{14}C that remained in the treated leaflet (TL) ranged from 98% 1 HAT to 76% 10 DAT.

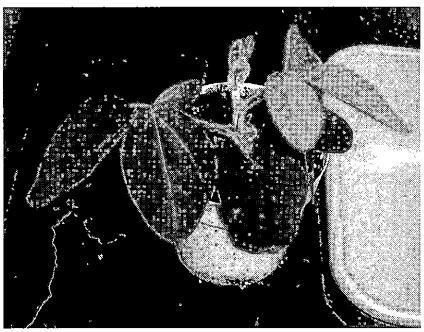
Harvest Time after application	Amount of 2,4-D applied (mg/plant)
1 hr	2.24
6 hrs	1.6
24 hrs	1.9
48 hrs	3.22
6 days	0.78
10 days	0.86

Table 1. Amount of 2,4-D applied to V3 soybean plants.

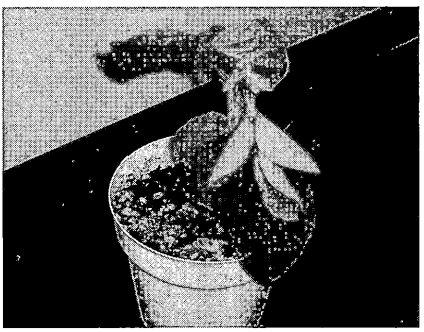
Time After Treatment	Severity of Symptoms[1]
1 hr	2
6 hr	2
24 hr	3
48 hr	3
6 days	6
10 days	8

[1]Scale of 1-10 with 10 being dead plants and 1 showing no injury.

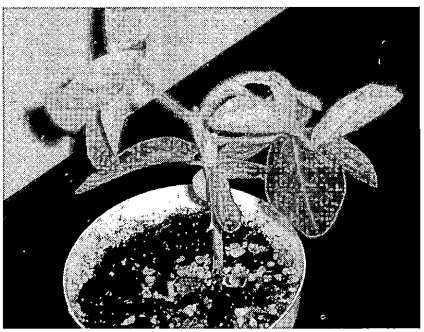
Table 2. Severity of soybean rating of injury symptoms that were observed in V3 soybean plants at corresponding harvest times.



1 HAT - injury severity 2



48 HAT – injury severity 3



10 DAT – injury severity 8

Figure 1. Severity of 2,4-D injury symptoms to a soybean plant at the V-3 stage, 1 hour after treatment (HAT), 48 HAT, and 10 days after treatment (DAT).

As time to harvest increased more of the 2,4-D was translocated to other parts of the plant. The areas of next highest concentration of ^{14}C was plant material above the treated leaf, the amount of ^{14}C that was in the area above the treaded leaf ranged from 0.8% 1 HAT to 10% 10 DAT. The remaining plant parts had very little ^{14}C in them, even 6 and 10 DAT.

METABOLISM AND CONJUGATION OF 2,4-D

The use of ^{14}C as a tracer for a nonlabelled chemical is an indication of the uptake and translocation patterns for the chemical. The question that needs to be answered is "Is the ^{14}C in the original herbicide form or has the herbicide been changed into a different chemical?" The process of changing the original chemical into a metabolite (breakdown of a parent compound to a smaller product) or a conjugate (adding a plant product to the original parent compound resulting in a larger product) can be accomplished several ways. Plants have been shown to join 2,4-D with a sugar, tripeptides, or single amino acids to form conjugates (Feung et al. 1972). In most cases, the joining of these compounds with 2,4-D turns the original chemical into an inactive compound. 2,4-D has also been shown to be metabolized in plants by undergoing B-oxidation (removal of the 2C chain of the carboxyl group) which also leads to a nonphytoxic product (Loos 1975). In the injury data, the plants that were harvested 10 DAT showed more severe symptoms than the plants harvested 48 HAT. This leads one to believe that after 10 d the chemical is still able to cause harm to the plant, therefore metabolism and/or conjugation are not likely to be occurring.

Thin Layer Chromatography (TLC) was used to evaluate the remaining ^{14}C in the plant and determine if the ^{14}C recovered was 2,4-D, or some other form. The amount of radioactivity in the aliquots of extract was too low to be detected by this method, except in the treated leaflet. Therefore, only results from the treated leaflet are presented.

	Total Applied		Partitioning of ^{14}C in Plant			
Time after Application	Leaf Surface	Uptake	Treated Leaflet	Remaining Trifoliate	Above Treated Leaf	Below Treated Leaf
	% of ^{14}C applied		% of ^{14}C taken up			
1 hr	57.5	42.5	96.5	0.6	2.5	0.5
6 hr	45.2	54.8	96.8	0.9	0.4	1.9
24 hr	35.4	64.6	86.2	3.2	2.7	7.9
48 hr	34.4	65.6	81.8	1.3	2.5	1.4
6 day	26	74	92.6	2.4	2.5	2.6
10 day	35.3	64.7	75.9	5.3	10.7	8.2

Table 3. Partitioning of ^{14}C in a V3 soybean plant from 1 hour to 10 days after application of ^{14}C-2,4-D to the middle leaflet of the 2nd trifoliate leaf.

A reproduction of the TLC plate is shown in Figure 2. The first lane is an aliquot of ^{14}C-2,4-D. The other lanes were spotted with treated leaf extract from each harvest time. The light spots in the lanes represent where the radioactive compounds are located. In the control lane only 2,4-D parent material is present. It moved the most in the developing solution, indicating that it was the most benzene soluble; the Rf value was calculated to be 0.55. This Rf value correlated with the Rf value reported by Feung et al. (1972). They used a TLC developing solution that contained 1-butanol-95%, ethanol-3 N ammonium hydroxide (4:1:5), and the Rf value found for 2,4-D was 0.42. In this study any light spots that had an Rf value of 0.55 would be assumed to be 2,4-D parent material.

The plants that were harvested 1 HAT and 6 HAT had almost 100% of the ^{14}C remaining as parent compound (Table 4). As the time the 2,4-D was in the leaf increased, the amount of chemical that changed into another form increased. Plants that were harvested 24 HAT had only 30% of the ^{14}C labeled 2,4-D remaining as parent material. The remaining ^{14}C was found at an Rf value of 0.32. The lower Rf value for this compound indicates that this form was more soluble in methanol than parent 2,4-D since methanol moved up the plates more slowly than benzene (Eastin 1986). The amount of metabolite remained about the same from 24 HAT to 6 DAT with an increase at 10 DAT. Others have reported water soluble forms of 2,4-D-based chemicals in soybean leaf, root, and cotyledon callus tissue (Feung et al. 1972; Davidonis et al. 1980), although these studies were not done on intact plants. Feung et al. (1972) reported both a glucose conjugate and an amino acid conjugate formed in cotyledon callus tissue 24 HAT.

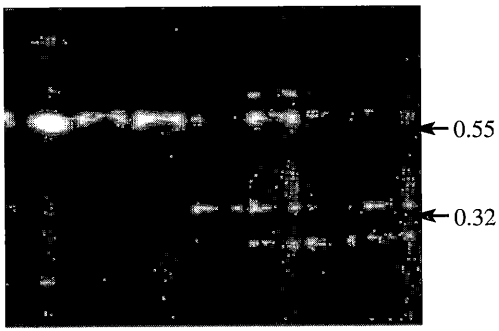


Figure 3. Thin layer chromatography of extract from the treated soybean leaflet. Bright spots indicate the presence of ^{14}C. Each lane is labeled with the time after application, Std is Standard 2,4-D, HAT is hours after treatment and DAT is days after treatment. The Rf values are 0.55 for the 2,4-D standard and 0.32 for the metabolite.

Davidonis et al. (1980) reported that 48 hrs after incubation root callus tissue did not metabolize or conjugate 2,4-D.

Cotyledon callus tissue formed 33% amino acid conjugates with the remainder staying as 2,4-D parent compound; and in the leaf callus tissue, amino acid conjugates were minor metabolites with glucose conjugates being the major metabolite. Davidonis et al. (1980) also showed that the age and type of callus tissue played an important role in the type and speed of alternative 2,4-D products formed, with older soybean callus tissue having a faster conjugation rate.

Time After Treatment	% Remaining 2,4-D parent compound	% Changed to metabolite
	% of total ^{14}C applied per lane	
1 hr	100	0
6 hr	98	2
24 hr	30	70
48 hr	36	64
6 days	38	62
10 days	24	76

Table 4. The portion of ^{14}C that remained as parent compound 2,4-D (Rf value = 0.55) and the amount that was metabolized or conjugated into a more water soluble compound (Rf value = 0.32).

Although extracts of other tissues were used in TLC analysis, the amounts of radioactivity were not detected using the plate imager. A few ways to solve this problem would be to put on more radioactivity per lane by 1) adding more aliquots of the extract or 2) further concentrating the extract. Another method may be to allow more time for development in the plate analyzer. Since the analyzer is a shared piece of equipment, we tried to maximize the time for development without compromising other researchers' needs. Without the ^{14}C TLC data, it is not possible to know if the 2,4-D in the other tissue was parent material or the metabolite. This study also did not determine if the plants metabolize the 2,4-D before or after it is translocated.

CONCLUSIONS

This study showed that 2,4-D uptake by soybean ranged from 39% to 64% in a 10 day period. Most of the ^{14}C that was taken up by the plants stayed in the treated leaf with about 10% moving to the youngest plant tissue and 8% moving to older tissue. The

radioactivity did not remain as parent 2,4-D but was fairly rapidly changed to a more water soluble metabolite with only 30 to 40% remaining as 2,4-D after 24 HAT.

Andersen et al. (2004) reported that 6 d after application of 2,4-D to field grown soybeans, less than 20% of the original application rate was detected by gas chromatography/mass spectrophotometry (GC/MS). By 12 d after application, only 3% of the 2,4-D applied was detected (Andersen et al. 2004). The 2,4-D was extracted by alkaline extraction and then plant extracts were derivatized to a more volatile form so parent 2,4-D could be detected using GC and quantified using MS. This extraction method would not detect the more soluble metabolites reported in this study. Based on this research and evidence from the literature, the 2,4-D in the soybean plant was being conjugated into more water-soluble forms, either glucose or amino acid conjugates, or both. These reactions would decrease the 2,4-D concentration over time and are similar in time-step to what Andersen et al. (2004) observed. These data would explain why, at the end of the season, no or very little 2,4-D was detected in stems or leaf tissue, although injury symptoms were very evident. An acidic extraction that may break the glucose or amino acid bond, and change the metabolite back to parent acid, may yield a higher amount of 2,4-D in these plant tissues if further degradation does not occur.

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Ruminal and Plasma Responses in Dairy Cows to Drenching or Feeding Glycerol

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ABSTRACT

Four Holstein dairy cows (137 DIM, 60 kg milk/d) were used in a Latin square with 1-wk periods to evaluate the effect of methods of oral delivery of glycerol on ruminal VFA and plasma concentrations of glucose, ß-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), and insulin. All cows were fed only grass hay for ad libitum consumption during 12 h before the experiment. At the start of the experiment, time 0, all cows were fed 5 kg of cracked corn. Treatments administered at time 0 were: 1) control (C), no glycerol; 2) fed glycerol (F), 1 kg of glycerol solution (80% glycerol) added to the corn; 3) drench glycerol (D), 1 kg of glycerol solution in 1 L of water and delivered as oral drench; and 4) tube delivery of glycerol (T), 1 kg of glycerol solution in 9 L of water and delivered into the rumen via an esophageal tube. Blood samples were collected at -1, -0.5, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after administering glycerol. Rumen samples were collected at 0, 2, 4, and 6 h. After administration of glycerol, concentrations of acetate decreased in rumens of cows while propionate and butyrate were increased by glycerol with peak concentrations at 4 h. Concentrations of glucose were increased in plasma of D and T compared with C, reaching peak concentrations at 1.5 and 3 h for D and T, respectively. Glucose response expressed as area under the curve (AUC) over baseline for 6 h was greater for D and T compared with C. Insulin concentrations in plasma were increased for D and T reaching peak concentrations at 1.4 and 1.1 h for D and T respectively. The 6-h AUC for insulin concentrations were greater for D and T than for F and C. The BHBA was increased in plasma of D, T, and F compared with C, reaching peak concentrations at 2.5, 2.4, and 1.6h for D, T, and F, respectively. These data demonstrate that the ability of glycerol to increase plasma concentrations of glucose and insulin is dependent upon rapid delivery.
Keywords: ß-hydroxybutyrate, dairy cows, glucose, glycerol

INTRODUCTION

Ketosis is an ongoing problem in high producing diary cows. Ketosis usually occurs between the second and seventh week of lactation when metabolic priority is given to the demands of milk production and appetite is often limited (Baird, 1982). Ketosis can be characterized by a drop in blood glucose and the subsequent elevation of blood ketone bodies and NEFA as fat stores are mobilized for energy (Schultz, 1968). One of the most

common methods of ketosis treatment is to administer glucogenic precursors, such as propylene glycol, through a drench. It has been known for some time that glycerol serves as an equal or better treatment for ketosis than common drenches like propylene glycol. (Johnson, 1955) The problem with glycerol has always been that the high cost eliminates it as a viable alternative for most producers.

With the advent of the biodiesel industry, of which glycerol is a byproduct, glycerol may become affordable for the average producer as a means of ketosis prevention/ treatment when delivered as an oral drench (Crandall, 2004). The question still remains as to the best method of delivery. It would be ideal to simply feed the glycerol and thereby avoid the need to provide it as a drench which is both labor intensive and stressful to the animal. The objective of this study was to determine whether feeding glycerol would provide the same positive effects as using it as a drench.

MATERIALS AND METHODS

Cows and Sampling

Four multiparous, lactating, Holstein cows (137 DIM, 60 kg milk/ day) were selected in July 2003 at the South Dakota State University Dairy Research and Training Facility. (Brookings, SD) The cows were used in a Latin square with 1-wk periods. Using protocols approved in accordance with SDSU IACUC policyCows were housed in individual tie-stalls, and fed ad libitum grass hay for 12 h prior to the start of the experiment to try to induce a mild ketosis. At the start of the experiment all cows were fed 5 kg of cracked corn as well as their assigned treatment. The treatments were: 1) control (C), no glycerol; 2) fed glycerol (F; West Central Soy, Ralston, IA), 1 kg of glycerol solution mixed with the cracked corn; 3) drench glycerol (D), 1 kg of glycerol solution mixed with 1 L of water and delivered as an oral drench directly into the oral cavity; 4) tube delivery of glycerol (T), 1 kg of glycerol solution mixed with 9 L of water and delivered directly into the rumen by a Cattle Pump System esophageal pump (The McGrath Company McCook, NE). The composition of the glycerol solution was 80.2% glycerol, 11.5% salt 6.6% water, and 1.3% methanol (DeFrain, 2004).

At 14 h prior to treatments, jugular catheters (Angiocath, Becton Dickinson and Co., Franklin Lakes, NJ) were inserted to assist in the collection of serial blood samples. Blood samples were collected into evacuated tubes containing K-EDTA (Becton Dickinson and Co., Franklin Lakes, NJ) at –1, -.5, 0, .25, .5, .75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h relative to time of administration of glycerol, and immediately placed on ice. Plasma was separated by centrifugation (American Scientific Products, McGraw Park, Ill.) at 5,800 x g for 10 minutes and stored at -20°C for later analysis. Rumen fluid was collected at 0, 2, 4, and 6 h relative to time of administration of glycerol by applying vacuum pressure to an esophageal tube fitted with a suction strainer. (DeFrain, 2004) One milliliter of 0.5 M sulfuric acid was mixed with a 10 ml sample of rumen fluid and the sample was frozen at -20°C for later analysis.

Laboratory Analysis

Plasma samples were thawed and concentrations of glucose were determined using glucose oxidase (Sigma Kit #315, Sigma Diagnostics, St. Louis, MO) according to the procedures of Trinder (1969). Concentrations of ß-hydroxybutyrate (BHBA) were determined (Sigma Kit 310-A, Sigma Diagnostics, St. Louis, MO) following the methods of Williamson et al. (1962), and concentrations of nonesterified fatty acids (NEFA) were determined using a colorimetric assay (NEFA-C Kit, Wako Chemicals, Richmond, VA), following modifications by Johnson and Peters (1993). Insulin was quantified by solid-phase radioimmunoassay. (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA)

For determination of VFA, rumen fluid samples were thawed and centrifuged at 32,000 x g for 20 min. (Jouan Inc. Winchester, VA). The concentrations of individual VFA were determined by gas chromatography (model 6890. Hewlett-Packard) using 2-ethylbutyrate as an internal standard in a 2-m glass column packed with GP 15% SP-1220/1% H3PO4 on Chromosorb w AW (Supelco Inc., Bellefonte, PA). Helium was used as the carrier as at a flow rate of 20 ml/min, and the temperature was isothermal at 200°C for injector, column and detector.

Statistical Analyses

Plasma data were analyzed as repeated measures using PROC MIXED of SAS software, version 8.1 (1999). For each variable, cow, period, and treatment were subjected to 5 covariance structures: variance components, autoregressive order one, compound symmetry, toeplitz, and unstructured. The structure yielding the Akaike's information criteria closest to 0 was used. For all metabolites, the model included treatment, period, time, and the treatment x time interaction. Plasma response data for glucose, BHBA, and insulin, included time to peak, peak height, and area under curve (AUC) and were calculated by integration of the first 6 h of data using Microcal Origin version 6.0. For calculation of response data, the mean values of the three samples collected prior to and including time 0 were used to determine baseline. Concentrations during the first 6 h that were greater than baseline were used to calculate AUC. Response data was analyzed with treatment and period as main effects.

Rumen VFA data, including acetate, propionate, butyrate, acetate:propionate, butyrate:propionate, and acetate+butyrate:propionate, were analyzed as repeated measures using PROC MIXED of SAS software, version 8.1 (1999). The model for rumen VFA included treatment, period, time, and the treatment x time interaction. Preplanned contrasts for comparison of all data included: 1. glycerol vs. control, 2. feed vs. forced, and 3. drench vs. tube.

Results and Discussion

DeFrain, et al. (2004) established the current knowledge available on feeding glycerol to transition cows. This research did not demonstrate the increases in blood glucose described by earlier researchers who delivered glycerol via esophageal drench (Johnson, 1955; Goff and Horst, 2001). The current experiment was able to directly compare methods of delivery as a possible explanation for inconsistencies.

Plasma glucose concentrations are illustrated in Figure 1. Peak glucose concentrations for all treatments occurred between 0.75 and 3h post feeding. Peak glucose concentrations were 77.4, 91.6, 79.0, and 90.0 mg/dl ($P < 0.01$) for C, D, F, and T respectively. The significant increase in plasma glucose for drenching and tubing is in agreement with Goff and Horst (2001). Contrasts for glycerol vs. none and fed vs. forced glycerol were significant ($P < 0.01$).

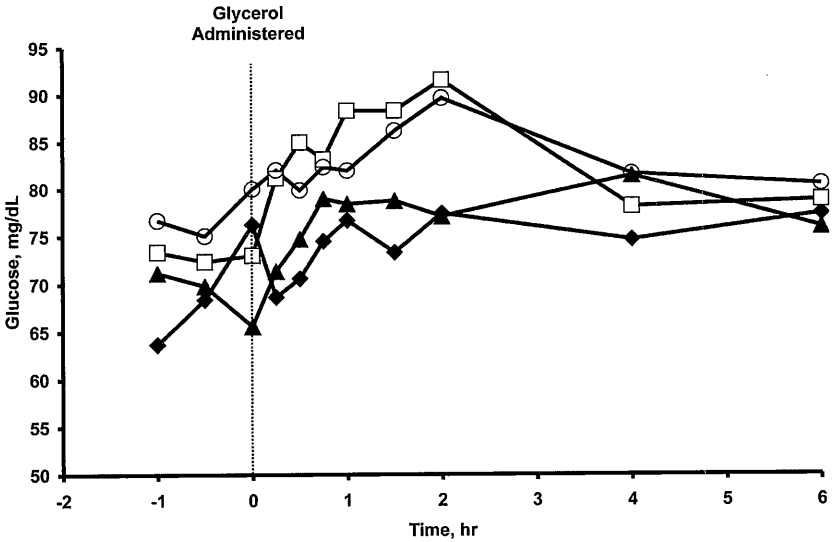


Figure 1. Concentrations of glucose (pooled SEM = 4.21) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

The 6-h response curves for plasma glucose are in Table 1. Peak height was increased significantly by glycerol supplementation, and again by forcing glycerol vs. feeding it ($P = 0.04$ and $P = 0.03$ for contrasts 1 and 2 respectively). Area under the response curve was increased ($P = 0.05$) by glycerol supplementation, and there was a tendency for further increase ($P = 0.08$) by delivering glycerol as either a drench or by espohageal tube. The slight increase in plasma glucose associated with feeding glycerol is in contrast with DeFrain, et al. (2004). This could possibly be explained by feeding glycerol in a concentrate vs. feeding glycerol as part of a total mixed ration.

	Treatment					Contrast		
	Control	Drench	Feed	Tube	SEM	Glycerol	Feed v. Forced	Drench v. Tube
						P<		
Glucose								
TP[1], h	2.9	1.5	1.6	3.0	0.8	0.38	0.44	0.24
PH[2], mg/dl	5.8	21.0	8.0	18.5	3.9	0.04	0.03	0.62
AUC[3],(mg x h)/dl	9.5	54.6	23.7	58.2	13.8	0.05	0.08	0.85
Insulin								
TP, h	2.2	1.5	1.0	1.1	0.8	0.29	0.80	0.83
PH, mg/dl	27.6	96.7	29.3	114.8	18.8	0.03	< 0.01	0.44
AUC,(mg x h)/dl	80.2	244.5	67.1	270.1	47.6	0.05	< 0.01	0.66
BHBA								
TP, h	1.2	2.5	1.6	2.4	0.5	0.12	0.2	0.86
PH, mg/dl	1.3	1.3	2.2	3.0	0.4	0.16	0.94	0.03
AUC,(mg x h)/dl	4.0	7.8	5.5	8.4	2.8	0.34	0.48	0.87

1 TP = Time to Peak; 2 PH = Peak Height; 3 AUC = Area Under Curve

Table 1. Plasma metabolite responses in cows treated with glycerol by adding to diet, drenching, or by esphogeal tube.

Plasma insulin also significantly increased ($P < 0.01$) with glycerol supplementation (Figure 2). Plasma insulin concentrations reached peak levels for all treatments between 1 and 2 h post feeding. Peak plasma insulin levels were 73.4, 144.9, 78.0, and 136.5 pg/ml for C, D, F, T respectively. These results, unlike the plasma glucose concentrations, agree with DeFrain, et al. (2004) for feeding glycerol. Peak height (Table 1) was increased significantly by glycerol supplementation, and again by forcing glycerol vs. feeding it ($P = 0.03$ and < 0.01 for contrasts 1 and 2 respectively). Area under the response curve was also increased by glycerol supplementation ($P = 0.05$) and again by either drenching or tubing glycerol ($P < 0.01$).

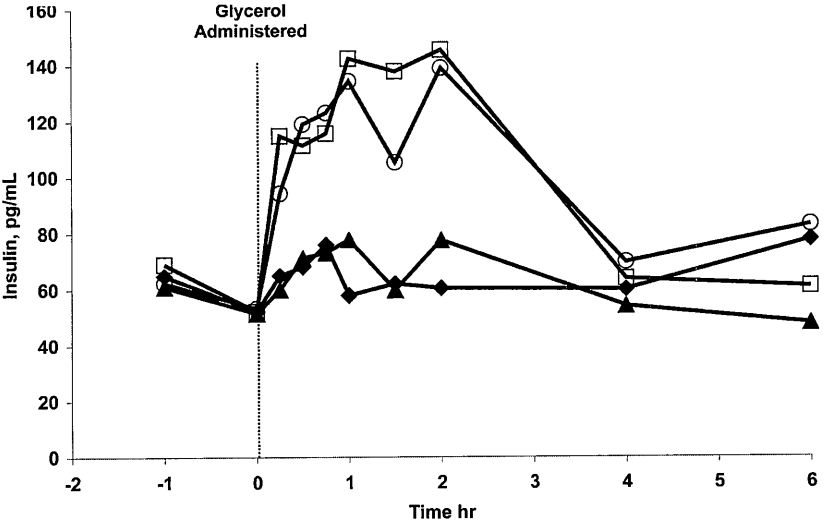
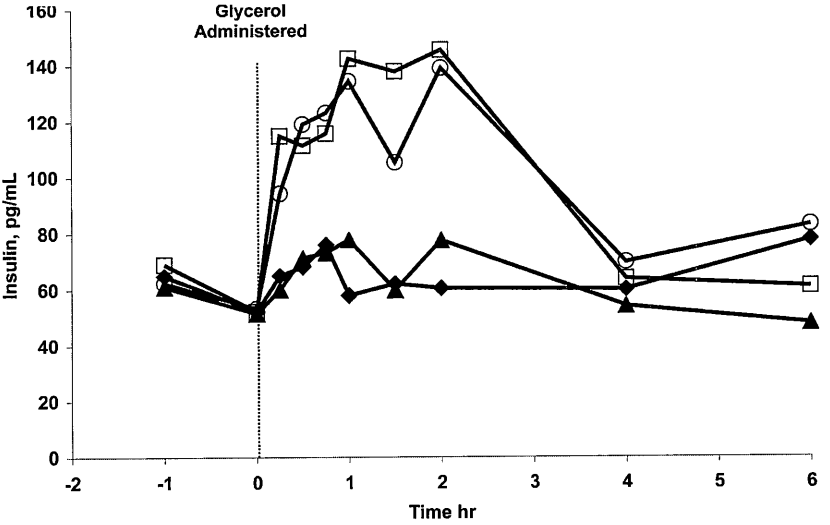


Figure 2. Concentrations of insulin (pooled SEM = 17.02) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

Plasma BHBA (Figure 3) was also significantly increased by glycerol supplementation. ($P < 0.01$). Peak plasma BHBA levels were 4.81, 6.98, 6.54, 7.04 mg/dl for C, D, F, and T, respectively. The greater increase of plasma BHBA for feeding glycerol vs. drenching or tubing glycerol as compared with plasma glucose or insulin is in agreement with DeFrain et al. (2004), and could possibly be explained by the significant increase of butyrate in the rumen (Figure 7). Peak height of BHBA (Table 1) over baseline values was greatest for tubing glycerol ($P = 0.03$ for contrast 3).



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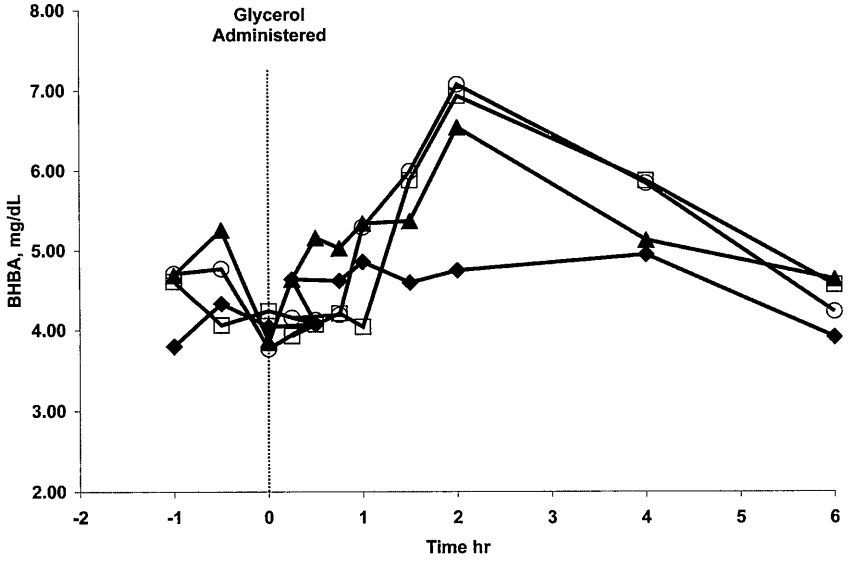

Figure 3. Concentrations of BHBA (pooled SEM = 0.86) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

Concentration of NEFA in plasma (Figure 4) was significantly decreased by glycerol supplementation ($P < 0.01$). Plasma NEFA levels at nadir were 530, 260, 380, and 230 μEq/L for C, D, F, and D, respectively. This could be attributable to a greater availability of plasma glycerol for the esterification FA. Plasma glycerol was not quantified.

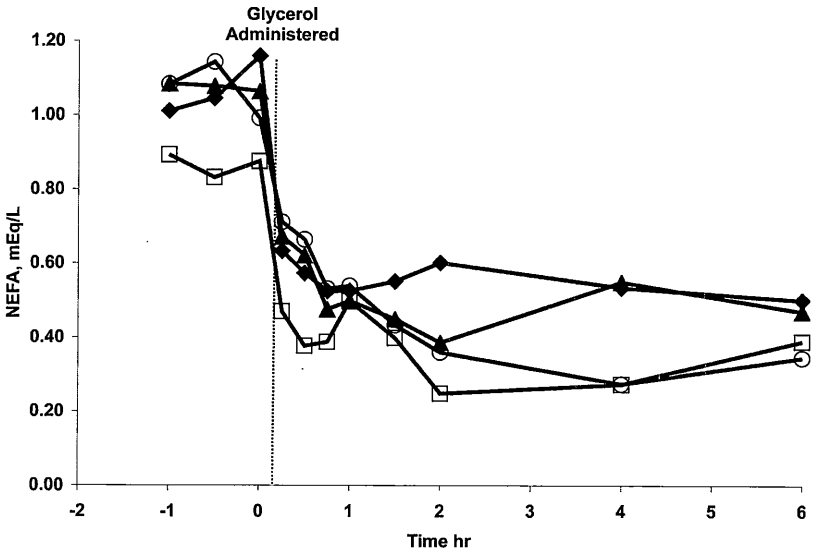

Figure 4. Concentrations of NEFA (pooled SEM = 0.14) in plasma of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

Glycerol supplementation significantly decreased ($P < 0.01$) ruminal acetate production (Figure 5) and tended to decrease acetate further by drenching and tubing ($P = 0.07$). Ruminal propionate production was significantly increased ($P < 0.01$) by glycerol supplementation (Figure 6) and drenching and tubing exhibited greater response than did feeding ($P = 0.02$). Ruminal butyrate production was also significantly increased ($P < 0.01$) by glycerol supplementation (Figure 7) and response was greater by drenching and tubing compared with feeding ($P = 0.05$). These data are in agreement with other research (Fischer et al., 1971; Rémond et al., 1993; Khalili et al., 1997; Schröder and Südekum, 1999) which described an increase in ruminal fermentation to butyrate associated with glycerol supplementation. This increase in fermentation to butyrate may also explain the increase in plasma BHBA in the presence of an increase in plasma glucose, as butyrate is extensively metabolized to BHBA by the rumen epithelium before delivery to circulation (Weigand, 1975).

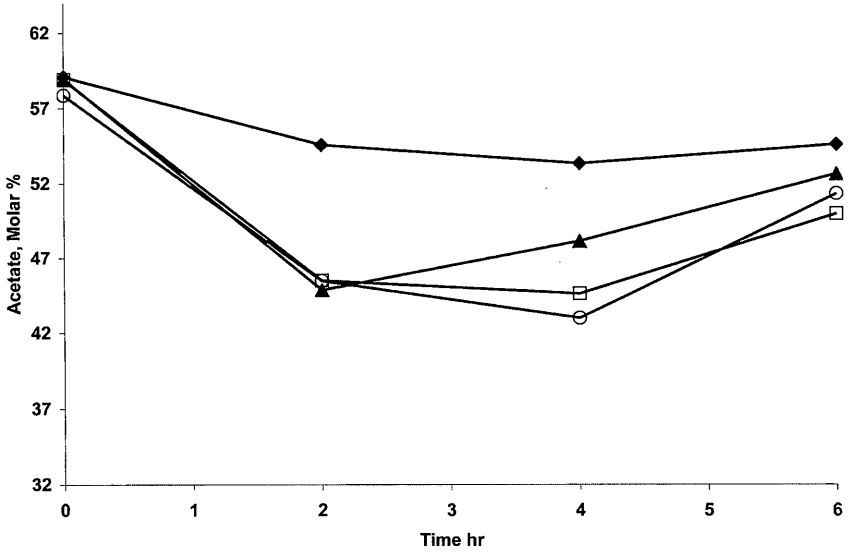

Figure 5. Molar percentages of acetate (pooled SEM = 1.54) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

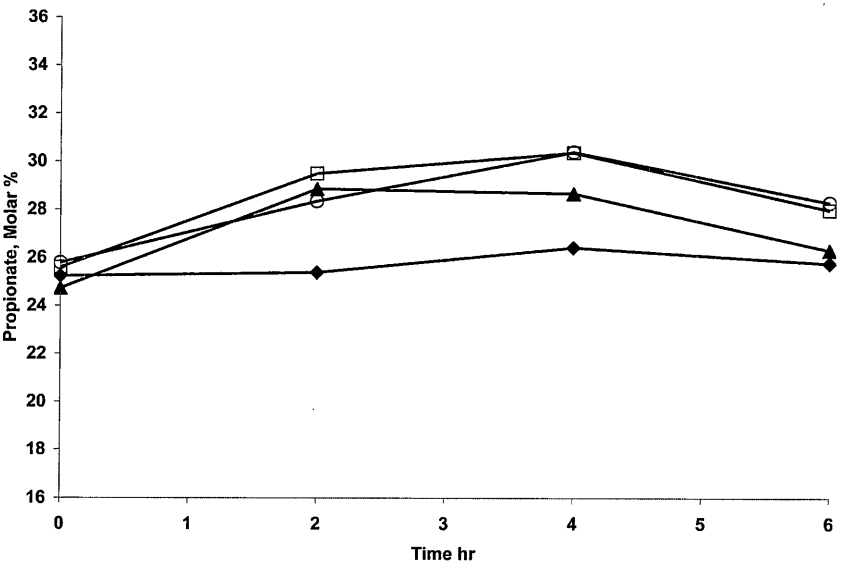

Figure 6. Molar percentages of propionate (pooled SEM = 0.86) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

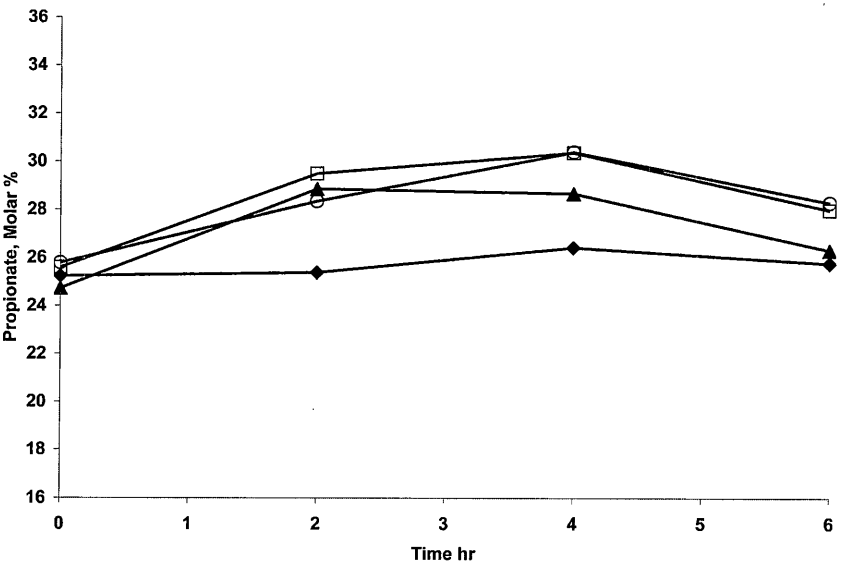

Figure 6. Molar percentages of propionate (pooled SEM = 0.86) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

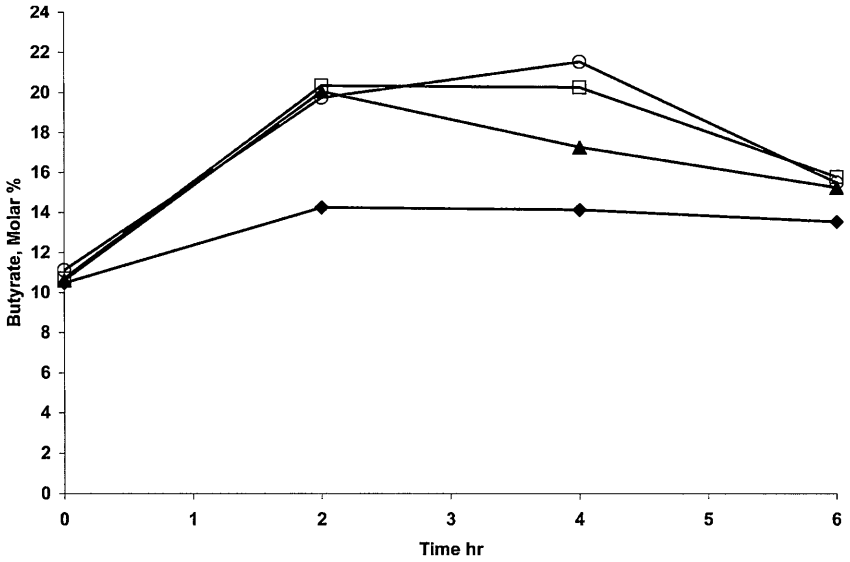

Figure 7. Molar percentages of butyrate (pooled SEM = 0.96) in rumen fluid of cows receiving glycerol via: No glycerol (C, diamonds), glycerol via drench (D, open squares), fed glycerol (F, triangles), and tubed glycerol (T, open circles).

CONCLUSIONS

Providing the cow with glycerol by drenching or tubing increases plasma glucose, insulin, and BHBA. Feeding glycerol increases plasma BHBA without increasing plasma glucose or insulin. Feeding glycerol also leads to increased ruminal fermentation to propionate and butyrate, and decreased fermentation to acetate. Increased fermentation to butyrate can lead to increased blood ketone bodies, BHBA, without a concurrent increase in blood glucose concentrations, which could lead to an increased likelihood of ketosis in dairy cows. It is the conclusion of these researchers that inclusion of glycerol in the feed may not be the best treatment or preventative for ketosis; however, the benefits of feeding glycerol to the lactating animal in a positive energy balance have not been adequately defined.

ACKNOWLEDGMENTS

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Shearing Sheep to Improve Growth Performance

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ABSTRACT

The purpose of this study was to determine if average daily gain (ADG) is improved in shorn lambs versus non-shorn lambs in the summer months in the upper Midwestern United States. Forty-nine purebred Hampshire and Columbia ram (n = 10 Hampshire and 4 Columbia) and ewe (n = 22 Hampshire and 13 Columbia) lambs were grouped by breed, sex and initial weight (49.6 ± 1.6 kg) into shorn (n=26) and non-shorn (n=23) groups. After shearing (3 June 2004), shorn sheep had approximately 0.2 cm of wool-cover. Lambs were weighed 1, 29, and 57 days following shearing. During the first 28 day period following shearing (period 1), there was no difference in average daily gain between shorn and non-shorn lambs (0.36 ± 0.02 vs 0.37 ± 0.02 kg/day, respectively; P=0.8289). In the second 28 day period (period 2), shorn lambs had a greater average daily gain than non-shorn lambs (0.46 ± 0.03 vs 0.42 ± 0.03 kg/day, respectively; P=0.0362). Period 2 had greater mean (20.56 vs. 17.78 oC, respectively), minimum (8.67 vs 6.72oC, respectively), and maximum (31.94 vs. 32.5oC, respectively) ambient air temperature and greater mean humidity (73.97 vs. 68.2 %, respectively) than period 1. The calculated thermal heat index (THI) was also greater in period 2 than period 1 (66.9 vs. 62.24, respectively). These data indicate that shorn lambs grow more rapidly than non-shorn lambs during periods of elevated temperature, humidity, and THI.

INTRODUCTION

Heat stress in lambs can result in reduced feed intake, feed efficiency and live weight gain (Ames and Brink 1977; Bhattacharya and Hussain 1974; Dixon, Thomas et al. 1999). Shearing is method by which producers attempt to reduce heat stress in growing or finishing lambs. Lambs are recommended to be shorn early in the feeding period to improve feed consumption, gain, and efficiency of feed conversion, especially in warm weather (Benson 2002). Research has supported this recommendation indicating shorn sheep consume more hay and concentrates than non-shorn sheep under heat stress conditions (24-44°C; da Costa, da Silva et al. 1992), and shorn lambs have higher apparent crude protein digestibility than non-shorn lambs (Horton 1981). Shorn sheep also have a reduced respiration rate compared to non-shorn sheep at 20°C (Phillips and Raghavan 1970; Horton 1981). In more moderate temperatures (ranging from 6 to 25°C),

shearing resulted in reduced feed intake in all sheep for at least 2 days, and persisted for three weeks in sheep which lost weight following shearing (Donnelly, Lynch et al. 1974).

Early studies demonstrated minimum metabolism occurred at temperatures ranging from 24-27°C in closely shorn sheep and energy retention became negative at temperatures below 15°C (Graham, Wainman et al. 1959). In a companion study, Blaxter et al. observed sheep with 2.5 cm of wool had a thermoneutral zone that ranged from approximately 12 to 31°C (Blaxter, Graham et al. 1959). Based on this early work, lambs which are not shorn may perform better than shorn lambs at more moderate temperatures.

The purpose of this study was to determine if average daily gain (ADG) is improved in shorn lambs versus non-shorn lambs in the summer months in South Dakota.

MATERIALS AND METHODS

Purebred Hampshire and Columbia ram (n = 10 Hampshire and 4 Columbia) and ewe lambs (n = 22 Hampshire and 13 Columbia) were assigned to one of two treatment groups: shorn or non-shorn. Treatments were balanced for breed, sex, and initial weight (49.6 ± 1.6 kg). Shorn lambs (n = 26) were shorn on 3 June 2004 with a 13 tooth comb fitted on a three inch shearing machine with four point cutter (Oster Shearmaster®, Niles, Illinois, USA). After shearing, shorn lambs had approximately 0.2 cm of wool-cover. All lambs were weighed 1, 29, and 57 days following shearing (4 June 2004, 2 July 2004, and 30 July 2004, respectively). All lambs were fed a 12% crude protein lamb finishing ration consisting of 72.5% cracked corn, 15% commercial lamb protein supplement (Big Gain Lamb Finisher, Big Gain, Inc. Mankato, MN, USA), and 12.5% pelleted soybean hull available ad libitum via a self feeder. Water was also available ad libitum. All lambs were segregated by sex, and lambs of the same sex were maintained together in a dry lot with shelter available. Climatic information was recorded every 30 minutes using a Vantage Pro Plus Weather Station equipped with WeatherLink for Vantage Pro Software (Davis Instruments, Hayward, CA) located within 500 meters of the pens where the lambs were housed. The temperature-humidity index (THI) was calculated using the equation:

THI = 0.8DBT + RH x (DBT-14.4) + 46.4 where, DBT is dry bulb temperature (°C) and RH is relative humidity in decimal form (Thom 1959).

Effect of treatment, sex and treatment by sex interaction on ADG during the 28 day period prior to shearing, the 28 day from 4 June 2004 until 2 July 2004 (period 1), the 28 day period from 2 July 2004 until 30 July 2004 (period 2), and over the entire study was analyzed by ANOVA using GLM procedures of SAS.

RESULTS

Period 2 had a greater minimum, maximum, and mean temperature of ambient air and a greater mean humidity than period 1 (Table 1 and Figure 1). The mean hourly temperature fell below 15°C in period 1 for 7 hours in the morning, but did not fall below

15°C in period 2 (Figure 1). Furthermore, in period 2 the mean hourly temperature reached 24°C for 5 hours a day. The THI was never greater than 82 in period 1 or 2 (Figure 2). However, the mean, minimum and maximum THI was greater for period 2 than period 1 (Table 1 and Figure 2). Mean wind speed and solar radiation were lower in period 2 than period 1 (Table 1).

In the 28 days prior to shearing, there was no difference in ADG among treatment groups (P=0.27). During period 1, there was no difference in ADG between shorn and non-shorn lambs (Figure 3; P=0.8289). In period 2, shorn lambs did have a greater ADG gain than non-shorn lambs (Figure 3; P=0.0362). However, overall ADG did not differ between treatments (0.39 ± 0.02 kg/day for non-shorn lambs vs. 0.41 ± 0.02 kg/day for shorn lambs; P=0.21). As anticipated, males had greater overall ADG than females (0.47 ± 0.02 vs. 0.38 ± 0.01 kg/day respectively; P=0.0002). There was no treatment by sex interaction for any of the time periods (P>0.36).

DISCUSSION

During period 1, there was no difference in ADG between shorn and non-shorn lambs. Mean maximum daily air temperature in period 1 (23.2 ± 0.8°C) approached temperatures at which shorn sheep have been observed to have increased feed intake relative to non-shorn sheep (24-44°C; da Costa, da Silva et al. 1992). However, the mean minimum daily temperature (12.3 ± 0.6°C) was well below the temperature of minimum metabolism (24-27°C) and also below the temperature at which energy retention becomes negative (15°C) in closely shorn sheep (Graham, Wainman et al. 1959). Weight loss has been reported in closely shorn lambs fed 110% of maintenance at average daily temperatures below the temperature at which energy retention becomes negative (11.6°C); (White, Fernandez et al. 1999). In the present study shorn lambs likely had an advantage in heat tolerance during peak temperatures in period 1, but negative energy retention which likely occurred during the coldest part of the day resulted in no advantage for shorn sheep in terms of average daily gain during period 1.

In period 2, shorn lambs had a greater ADG than non-shorn lambs. Elevated ambient temperatures in period 2 compared to period 1 could contribute to this difference in growth performance. Mean maximum daily temperature (25.7 ± 0.7°C) was within the temperature range at which shorn sheep have been observed to have increased feed intake relative to non-shorn sheep (24-44°C; da Costa, da Silva et al. 1992), and also within the temperature of minimum metabolism (24-27°C) for closely shorn sheep (Graham, Wainman et al. 1959). The mean minimum daily temperature (15.4 ± 0.7°C) was also just above the temperature at which energy retention becomes negative (15°C) in closely shorn sheep (Graham, Wainman et al. 1959). Additionally, shorn lambs have been reported to have maximum ADG when housed at constant temperatures of 15°C (Ames and Brink 1977). The shorn lambs likely had an advantage in heat tolerance during the hottest part of the day which resulted in greater ADG gain than non-shorn lambs in period 2.

Additionally, the small amount of wool re-growth which would have occurred in the shorn lambs could have increased the shorn lambs' ability to tolerate cooler temperatures. Although wool growth would be expected to vary by breed, wool growth of 0.08 mm to 0.18 mm per day has been reported (Donnelly, Lynch et al. 1974). At that rate of wool growth, shorn lambs in this study would have had 0.38 cm and 0.66 cm of wool cover at the beginning of period 2. This amount of additional wool cover may have allowed the shorn lambs in period 2 to better tolerate minimum temperatures during the coldest part of the day.

In addition to ambient temperature, relative humidity can also impact animal performance and response to the environment. The THI is a derived statistic that was originally used to evaluate thermal effects in humans (Thom 1959). It has been used in livestock to estimate the combined impact of temperature and humidity on performance and serves as the basis for the Livestock Weather Safety Index (UDSC-ESSA 1970). In sheep, daily changes in vaginal and ear-canal temperatures are strongly correlated with changes in THI (Lowe, Cook et al. 2001). A THI of 74 or less is considered normal, 75 to 78 is alert status, 79 to 83 is danger status, and a THI equal to or above 84 is an emergency (LCI 1970). Although the THI in the current study never reached emergency status, the THI did rise into the alert and danger status in both period 1 and 2. The THI was in the alert or danger status for a greater amount of time in period 2 than period 1. In the present study shearing may have allowed the lambs in period 2 to better tolerate conditions when they reached the alert or danger categories resulting in increased ADG.

Although wind speed and solar radiation were lower in period 2 than period 1, these two factors likely did not influence ADG. Both the shorn and non-shorn lambs had free access to covered shelter which would negate the impact of intense solar radiation. This shelter likely also would have reduced an impact of wind speed on animal performance.

This research suggests that shearing lambs would be beneficial during the summer months when ambient temperatures and THI values are elevated. Under moderate climatic conditions, performance responses indicate no additional advantages.

	Ambient Temperature	Relative Humidity (%)	THI	Windspeed (km/h)	Solar radiation (W/m2)
	Mean ± std dev	Mean ± std dev	Mean ± std dev	Mean ± std dev	Mean ± std dev
Period 1	17.78 ± 5.14	68.2 ± 19.7	62.24 ± 7.04	5.92 ± 5.69	194.42 ± 203.59
Period 2	20.56 ± 4.74	73.97 ± 16.99	66.9 ± 6.71	4.75 ± 4.53	192.27 ± 202.81
Overall	19.26 ± 5.12	71.28 ± 18.53	64.73 ± 7.25	5.34 ± 5.18	193.27 ± 203.14

Table 1: Mean climatic data for period 1 and 2

Climate data was recorded every 30 minutes using a Vantage Pro Plus Weather Station equipped with WeatherLink for Vantage Pro Software (Davis Instruments, Hayward, CA) located within 500 meters of pens where the lambs were housed. THI =

0.8DBT + RH x (DBT-14.4) + 46.4 where, DBT is dry bulb temperature (°C) and RH is relative humidity in decimal form (Thom 1959).

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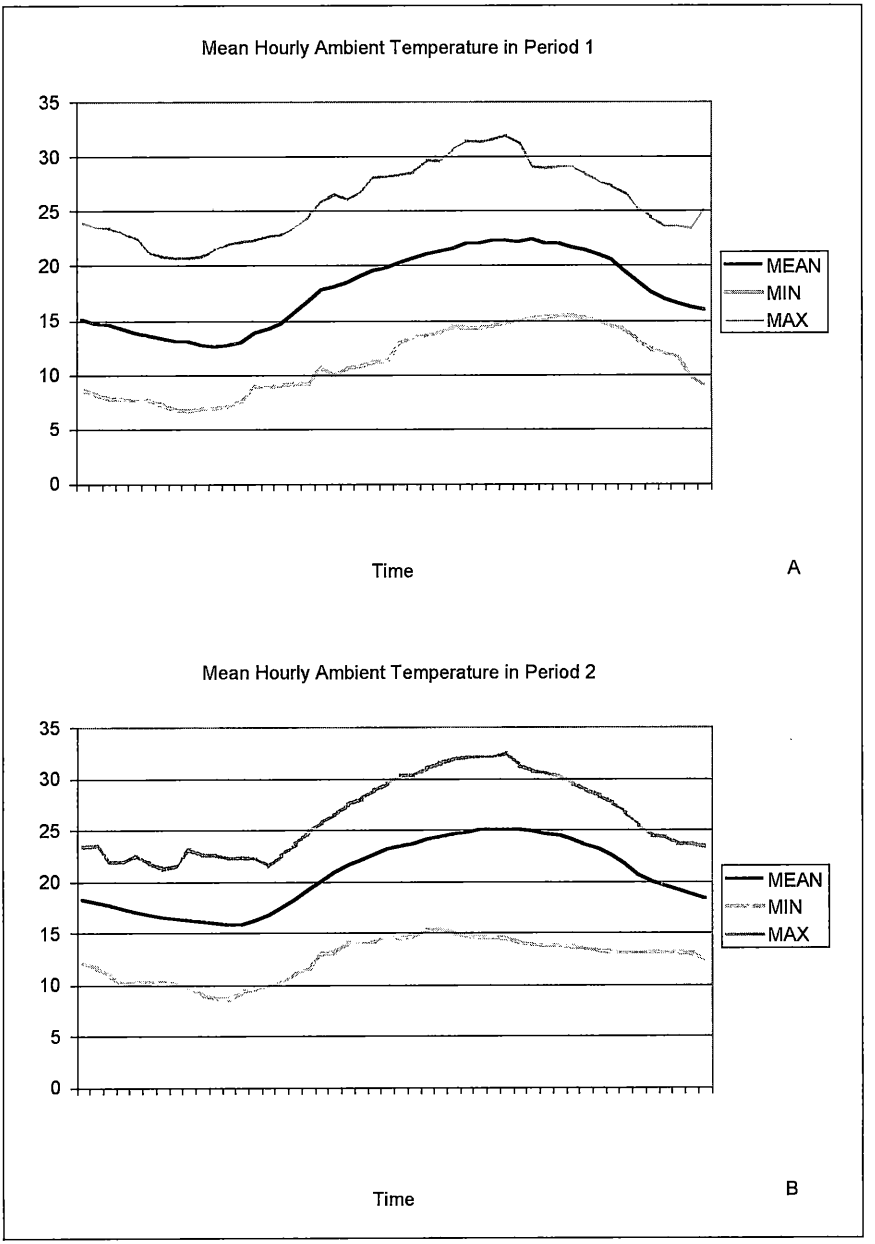

Figure 1. Mean, minimum, and maximum hourly ambient temperature for period 1(A) and period 2 (B).

Furniture Usage and Activity Budgets of Captive Black and White Ruffed Lemurs (Varecia variegata variegata) and Ring-Tailed Lemurs (Lemur catta) at Bramble Park Zoo, Watertown, South Dakota

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ABSTRACT

A behavioral study was conducted on the black and white ruffed lemurs and ring-tailed lemurs at Bramble Park Zoo in summer 2004 to determine if the furniture in the exhibit was sufficient to enable them to display their natural behaviors. The study was performed using a time sampling method of one minute, and ten hours of data was gathered. Both species of lemur spent a significant portion of the observed time resting, more so than has been found in wild populations of lemurs. The time spent foraging (.33% and 2.5% for the ruffed lemurs and 2.1% for the ring-tails) and the time spent displaying locomotion behaviors (1.6% and 7.0% for the ruffed lemurs and 4.45% for the ring-tails) were found to be lower in the captive lemurs compared to data for wild lemurs (~30-40% foraging and 17% locomotion behaviors). Black and white ruffed lemurs, which are primarily arboreal in the wild, spent a majority of time on the ground. The ring-tailed lemurs, a semi-terrestrial species, spent around 50% of the time in the tree and around 40% of time on the ground, which is similar to ring-tailed lemurs in the wild; however, most of that time was spent resting rather than foraging or displaying locomotion behaviors. Several ideas pertaining to furniture modifications and food presentation methods were suggested as a means to increase natural behaviors as well as decrease the amount of time spent resting.

INTRODUCTION/BACKGROUND

The black and white ruffed lemur (Varecia variegata variegata) and ring-tailed lemur (Lemur catta) are primates native to the island of Madagascar located off the southeastern coast of Africa. Lemurs are a type of primate known as prosimian, meaning "pre-monkey," indicating that these species are primitive versions of primates (Berger, 1985). While black and white ruffed and ring-tailed lemurs are in the same family, Lemuridae, and do share some behavior traits, there are distinctive differences in their natural behaviors.

Black and white ruffed lemurs in the wild are arboreal, spending most of their time in upper layers of the canopy. While the locomotion of these lemurs is more cautious compared with other lemur species, they are agile on the ground and in trees, walking or running on large branches and leaping between trees. Black and white ruffed lemurs are a crepuscular species and usually sleep for a large part of the day (Macdonald, 1984). Black and white ruffed lemurs can be seen "sunbathing" during the day, lying stretched out in the sunlight. Black and white ruffed lemurs are mainly frugivores, as 92% of their diet consists of fruit (Britt, 2000). This species also eats leaves and nectar as well (Britt and Iambana, 2003). Black and white ruffed lemurs display several unique postures when feeding, with the most interesting being their ability to hang upside-down by their feet to reach food on small branches (Konstant et al., 1994). In both black and white ruffed lemurs and ring-tailed lemurs, females are dominant over males, and there is usually one dominant matriarch in a group (Macdonald, 1984). Social interactions, such as grooming and vocalization, are very important in both species for establishing bonds within a group. All lemurs have a "tooth comb," located where the lower incisors are, as well as a "toilet claw," located on the second digit of the back foot, both of which are used for grooming. Black and white ruffed lemurs communicate mainly through vocalizations, and 12 different calls have been recorded in this species, many of which are alarmingly loud (McLennan and Pappas, 2003a).

Ring-tailed lemurs are the most terrestrial of all lemur species, frequently using the ground for traveling and foraging (Macdonald 1984). Ring-tailed lemurs are diurnal and sunbathe during the day in a meditation-like position with their arms outstretched. Wild ring-tailed lemurs spend a large portion of their day foraging and feeding on the ground, and can cover up to 900-1000 meters per day (Konstant et al., 1994). Ring-tailed lemurs rely on scent more than vocalizations for communication. All ring-tailed lemurs have scent glands on their wrists, chests, and feet, and males have an extra gland located in their armpits. While the females of this species can be fierce fighters, the males are less aggressive. Instead of fighting, males will rub their tails on their wrist glands and then wave their tail at their opponent, "throwing" their scent at them. This is known as a "stink fight" and is a unique behavior of the ring-tailed lemur (McLennan and Pappas, 2003b). There are also about 22 different calls that these lemurs make, including grunting, howling and purring (Macedonia, 1993).

Both lemur species are listed as endangered and many zoos and wildlife reserves are working to increase the numbers of these lemurs (Nowak, 1999). A central objective of zoos with primate species is to ensure that their exhibits are as naturalistic as possible, providing a replication of natural habitat, while at the same time enabling the public to view the animals. While size of the enclosure can be a factor in limiting certain types of natural behaviors as well as increasing stereotypic behaviors, the idea of functional space is more commonly considered. Functional space is "the space that animals use rather than the total amount of space (Beckley et al., 1994)." Many animals utilize more space in their enclosures than just the size of the cage, especially primate species. Placing furniture in an exhibit, which can include branches, ropes, swings, and rocks, can further increase existing functional space as well as increase natural behaviors of many different primate species, including lemurs.

For this study, furniture usage along with behaviors displayed by the two species of lemurs was examined. The purpose of the study was to determine if furniture in the cage allowed captive lemurs to exhibit behaviors normally found in wild lemurs. These two species were chosen for this experiment because the lemur cage did not seem to match with their behavioral needs. General information about each of the five lemurs studied includes (courtesy of the Bramble Park Zoo):

Phoebe (V. v. variegata)
- 29 year old female (all black face)
- Date of Birth: 5-19-1975
- Place of birth: Duke Primate Center
- Date of Acquisition by Bramble Park Zoo: 4-20-1987

Gwen (V. v. variegata)
- Phoebe's daughter (white on face)
- 17 year old female
- Date of Birth: 5-11-1987
- Place of Birth: Duke Primate Center
- Date of Acquisition by Bramble Park Zoo: 4-20-1987

Josh (L. catta)
- 8 year old male
- Date of Birth: 4-15-1996
- Place of Birth: St. Catherine's Wildlife Center
- Date of Acquisition by Bramble Park Zoo: 7-21-1999

Ossabaw (L. catta)
- 14 year old male
- Date of Birth: 3-22-1990
- Place of Birth: St. Catherine's Wildlife Center
- Date of Acquisition by Bramble Park Zoo: 7-21-1999

McQueen (L. catta)
- 6 year old male
- Date of Birth: 3-12-1998
- Place of Birth: St. Catherine's Wildlife Center
- Date of Acquisition by Bramble Park Zoo: 7-21-1999

STUDY AREA

I conducted this project during summer 2004 while on internship at the Bramble Park Zoo, an AZA accredited zoo in Watertown, South Dakota. Both lemur species were

housed together in one exhibit. There were two female black and white ruffed lemurs and three male ring-tailed lemurs living in the exhibit. The exhibit is connected to a building known as the Triplex (Figure 1), as are two other exhibits, both of which also house primates. While there is an indoor area for the lemurs, I focused only on the outdoor area. The outdoor exhibit consists of a circular section of grass surrounded by a thin circular area of cement. Vertical and horizontal bars surround the outer edges of the cage and there is a small section near the door where bars are used as roofing instead of the solid roof which covers the rest of the exhibit. In the grass portion of the exhibit, there are two large rocks which sit close together near the inside door, and a large tree with several branches reaching to the top of the exhibit. For this study, I grouped the tree branches based on size: one large limb, five medium limbs, and nine small limbs (Figure 2). There were also several locations on the tree where two branches split from each other and these areas were designated as a branched V.

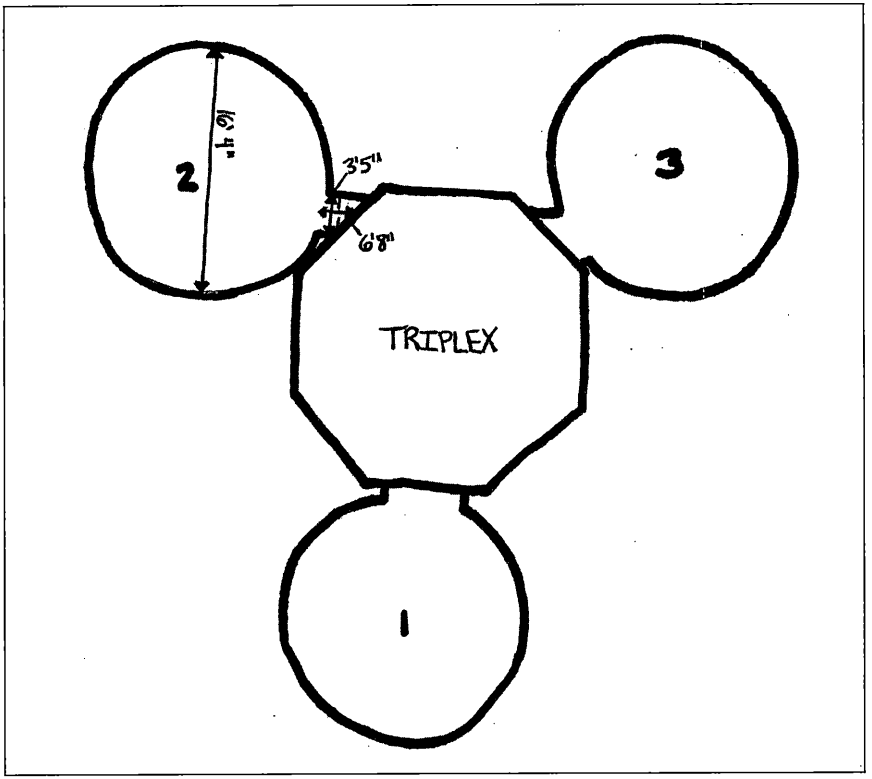

Figure 1. The Triplex layout at Bramble Park Zoo in Watertown, South Dakota. Area 2 is the black and white and ring-tailed lemur enclosure. Measurements of the circular area, which is the outdoor portion of the exhibit, are given.

Figure 2. Photograph of the lemur exhibit at Bramble Park Zoo in Watertown, South Dakota from a front view looking towards the Triplex building (in the background).

METHODS

I conducted a total of 20 observations, each lasting for 30 minutes and gathered a total of 600 minutes of data. The method of observation I used was scanning, or instantaneous sampling, where I devised a chart to record what each lemur was doing and what furniture they were using at a time interval of one minute. The observation periods were scattered throughout the day, ranging from as early as 6:30 A.M. to 8:30 P.M. to ensure that data was collected during the active periods for both lemur species.

During every 30-minute observation period, I would stand outside the lemur exhibit and observe which furniture they were on as well as what behavior they were displaying. Each minute, the behavior displayed by the five lemurs and the furniture they were utilizing was recorded. There were six broad categories of furniture (tree, ropes, ground, rocks, outer cage and inside). There were eleven broad categories of behaviors including laying/sleeping, sitting, eating, foraging, grooming, locomotion, drinking, fighting, vocalizing, scent marking, and urinating/defecating. The locomotion category was further categorized as walking, running, swinging, climbing, hanging and leaping. Each lemur was designated a letter and number: L1 was given to the older female black and white ruffed lemur, Phoebe, L2 was designated to her daughter, Gwen, and R1-3 were assigned for the ring-tailed lemurs. Because I was not able to tell the three male ring-tailed lemurs apart, they were randomly given a letter and number (R1-R3) each observation period, and then all of their data was pooled, for 1800 minutes of information. Following each observation, the data was counted and put into groupings of furniture used and behaviors displayed on that furniture.

RESULTS

All five lemurs spent a large portion of time resting (either laying/sleeping or sitting) during the study. L1 spent 77.7% of the time resting (Figure 3), while L2 spent 58.3% of the time resting (Figure 4). R1-R3 spent 43.8% of the time resting (Figure 5). Grooming (both self-grooming and reciprocal grooming) was the second most common behavior observed in both lemur species. L1 and L2 spent 7.2% and 7.0% of the time grooming. R1-R3 spent 22.1% of the observed time grooming. L2 spent 7.0% of the time displaying several locomotion behaviors, which included walking, leaping, climbing and hanging, and spent 5.2% of the time eating. R1-R3 displayed locomotion behaviors 4.5% of the time and spent 5.1% of the time eating. L1 displayed both locomotion and eating behaviors less frequently than the other lemurs, at 2.4% and 1.6%, respectively. Other behaviors observed included foraging/exploring, vocalizing, stink fighting and scent marking; however, the occurrence of these behaviors was low.

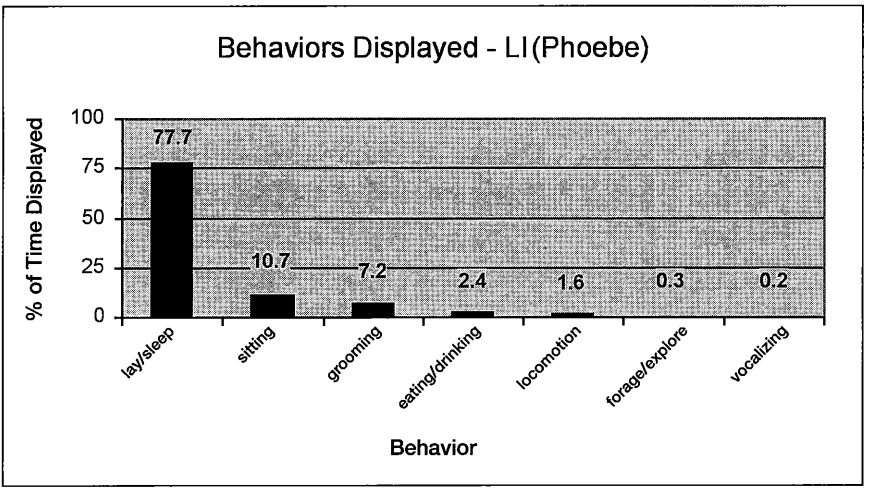

Figure 3. Percent of time specific behaviors were displayed by L1 (black and white ruffed lemur Phoebe (Varecia variegata)) at Bramble Park Zoo, summer 2004. A time sampling method of 1 minute was used to gather data.

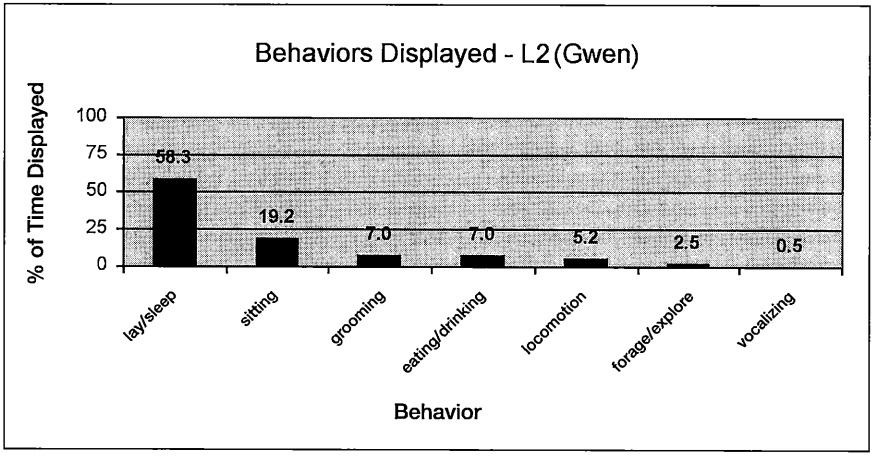

Figure 4. Percent of time specific behaviors were displayed by L2 (black and white ruffed lemur Gwen (Varecia variegata)) at Bramble Park Zoo, summer 2004. A time sampling method of 1 minute was used to gather data.

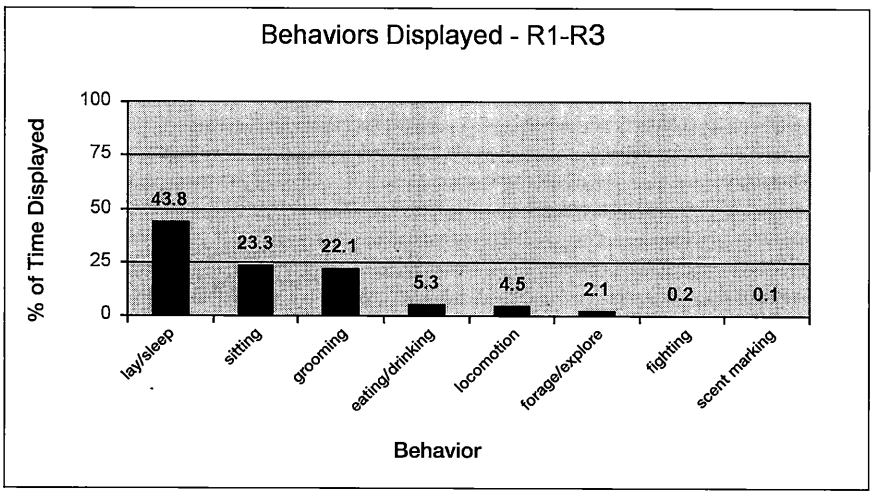

Figure 5. Percent of time specific behaviors were displayed by R1-R3 (ring-tailed lemurs (Lemur catta)) at Bramble Park Zoo, summer 2004. A time sampling method of 1 minute was used to gather data.

Wild black and white ruffed lemurs spend about 44% of their day resting, 30% feeding and foraging, 17% traveling, and 9% involved in social interactions, such as grooming, scent marking and vocalization (McLennan and Pappas, 2003a). Studies at Berenty Reserve in Madagascar found that wild ring-tailed lemurs spend about 31% of the day foraging, 50% resting (including grooming), and 13% traveling (Kieth-Lucas et al., 1999). Wild ring-tailed lemurs spend around 40% of their day on the ground, during which they are mostly active, either searching for food or traveling through their territory (McLennan and Pappas, 2003b).

L1 and L2 spent most of their time on the ground in the exhibit (54.6% and 70.8%) (Figure 6 & 7). L1 also spent a significant portion of time (41.5%) on a specific medium limb of the tree. The furniture used most by R1-R3 (Figure 8) was the tree (47.8% on the large limb), and only 30% of their time was spent on the ground. Each piece of furniture or area in the exhibit was utilized by the lemurs to some extent, except for the vertical rope, which was never used during the 600 minutes of observation time.

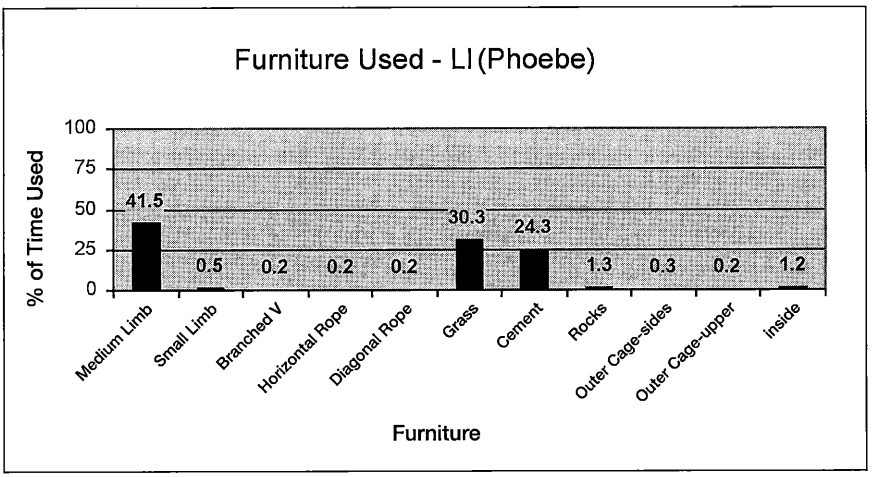

Figure 6. Percent of time exhibit furniture was used by L1 (black and white ruffed lemur Phoebe (Varecia variegata)) at Bramble Park Zoo, summer 2004. A time sampling method of 1 minute was used to gather data.

*Note: Phoebe was Inside 1.2% of the observation time

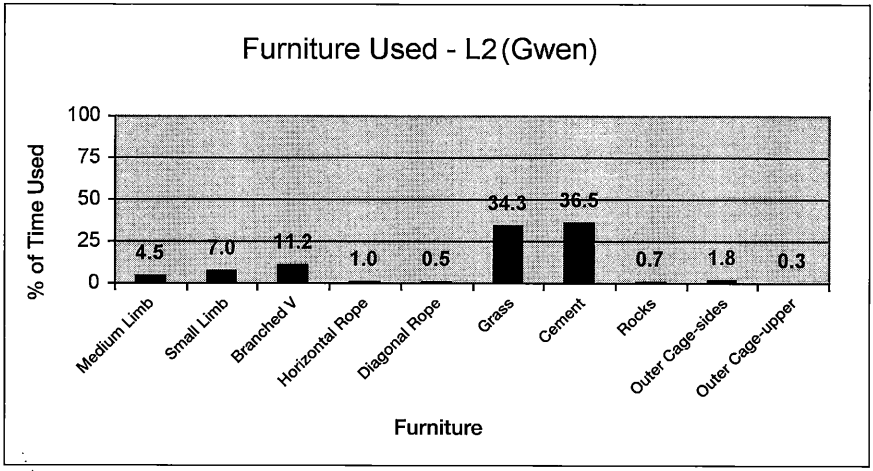

Figure 7. Percent of time exhibit furniture was used by L2 (black and white ruffed lemur Gwen (Varecia variegata)) at Bramble Park Zoo, summer 2004. A time sampling method of 1 minute was used to gather data.

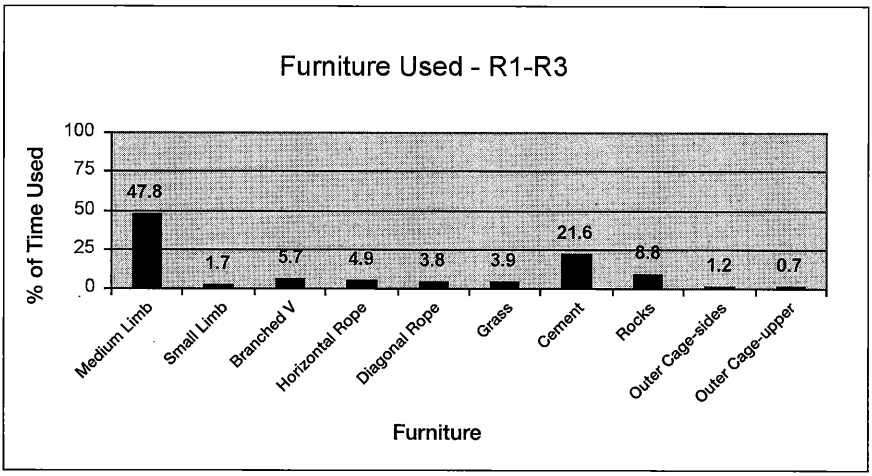

Figure 8. Percent of time exhibit furniture was used by R1-R3 (ring-tailed lemurs (Lemur catta)) at Bramble Park Zoo, summer 2004. A time sampling method of 1 minute was used to gather data.

DISCUSSION

Both lemur species spent most of the time resting, while only a small portion of their time was spent displaying locomotion behaviors and foraging or feeding behaviors. Wild lemurs generally spend more time feeding, foraging and traveling, and less time resting, than I observed for the captive lemurs at the zoo. L1 spent more time resting than the other lemurs; however, in her case this does not appear to be out of the ordinary given her age (29 years old). The average life span for this species of lemur is between 15-20 years in the wild and around 20 years in captivity (McLennan and Pappas, 2003a). L2 also spent a slightly more time resting than wild lemurs, which also may be due to her age (17 years). In comparison with the data for wild black and white ruffed lemurs, the foraging/feeding and traveling (locomotion) of the captive lemurs were considerably different. However, the time spent displaying social interactions (grooming) in L1 and L2 was similar to that of wild lemurs. For both L1 and L2, behaviors other than resting were displayed more often on the ground than in the tree.

R1-R3 spent a large percentage of time resting, but spent more time grooming than L1 or L2. In most cases, this grooming was reciprocal grooming between two or all three of the lemurs, unlike L1 and L2 in which the grooming was largely self-grooming. In comparison to data for wild ring-tailed lemurs, the captive lemurs spent more time resting (including grooming) and much less time foraging. The percentage of time spent moving around the exhibit (traveling) was also less than that in the wild lemurs. R1-R3 spent almost half of the study time on top of the one large tree limb (this limb was never used by the ruffed lemurs during the observational period), and around 30% of their time on the ground, which correlates fairly closely to data from the Duke Primate Center. However, only a small percentage of their time spent on the ground was spent foraging or moving around (locomotion behaviors).

CONCLUSION

The differences between the observed behaviors of the two captive species of lemurs compared to the activity budgets for these species in the wild indicate that changes in the layout of exhibit furniture could be made in an attempt to promote more natural behaviors. Several studies have shown that more natural environments, such as island exhibits, enable and encourage lemur species to exhibit more natural behaviors (Britt, 1997; Kieth el al., 1999; Kerridge, 1997). Currently, the only branches available to lemurs are on the tree and most extend to the top of the exhibit and are fairly similar in dimension. It may be beneficial to change the branches to a more complex and natural arrangement that mimics the three-dimensional complexity of a tree canopy. The feeding methods could also be altered to promote more natural behaviors, such as locomotion and feeding, since there is little ground area available in the exhibit. Generally at the zoo, food is placed in a pile on the grass or cement, which does not encourage much foraging behavior. Possible food presentation methods could include hanging individual food items or baskets of food from branches and ropes at various heights, hiding food in

crevices of branches and elsewhere in the exhibit, and placing or planting browse in the enclosure (Elder, Primate Zookeeper, St. Paul's Como Zoo, written communication). These ideas for furniture modifications and food presentation may be advantageous in promoting more natural behaviors in the two species of lemurs at the Bramble Park Zoo, and may be especially helpful in decreasing the amount of time spent resting.

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